

FINAL

**Report on Carcinogens
Background Document for**

Styrene

September 29, 2008



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

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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chemical and physical properties, analytical methods, production, use, and occurrence may come from published and/or unpublished sources.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>.

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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:

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens
U.S. Department of Health and Human Services
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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.

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Executive Summary

1 Introduction

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

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

8 Human Exposure

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

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

5 **Human Cancer Studies**

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

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

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

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

5 In the styrene-butadiene industry, the cohort studies are among the largest, with the
6 longest follow-up times. The principal methodological challenge is to separate the
7 potentially independent or synergistic effects of butadiene, a known human carcinogen,
8 which is highly correlated with styrene in this industry. Two independent (non-
9 overlapping populations) are available, a small cohort of 6,678 male workers at a rubber
10 tire manufacturing plant (a subset of the workers were engaged in the production of
11 styrene-butadiene and other rubbers) (McMichael *et al.* 1976a) and a larger cohort
12 established by Delzell and colleagues (Delzell *et al.* 1996, 2006) of 13,130 to 16,610
13 styrene-butadiene rubber industry workers from multiple plants in the United States and
14 Canada. The cohort established by Delzell includes most (but not all) of the workers from
15 two cohorts — a 2-plant cohort (Texas) (Meinhardt *et al.* 1982) and an 8-plant cohort
16 originally established by Matanoski and colleagues (United States and Canada) and
17 reported in a series of previous publications (7 of the 8 plants were included in the
18 Delzell cohort). Thus, there is considerable overlap between these populations. Two
19 nested case-control studies (Matanoski *et al.* 1997, Santos-Burgoa *et al.* 1992) of a single
20 group of cases with lymphohematopoietic cancers were available from the Matanoski
21 cohort. The Delzell cohort expanded the previous cohorts to include workers employed
22 from 1943 to January 1, 1991 and followed to 1998, whereas the earlier cohort included
23 workers employed until 1976 and followed until 1982. In addition, the individual study
24 populations were established by different procedures and exclusion criteria (which may
25 partly explain the lack of complete consistency in the number of study subjects across the
26 published studies) and often used different exposure assessments, selection of study
27 subjects, and types of analysis. Two types of analyses were conducted on the Delzell
28 cohort: external analyses reporting on standardized mortality ratios (SMRs) for the total
29 cohort or subsets of the cohorts for multiple cancers sites (Sathiakumar *et al.* 1998,
30 2005), and, secondly, internal analyses of relative risk (RR) estimates for quantitative
31 exposure to styrene and lymphohematopoietic cancers (Delzell *et al.* 2001, 2006,

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

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

12 *Lymphohematopoietic cancers*

13 Statistically significant increases were observed for all lymphohematopoietic cancers
14 combined and leukemia among rubber-tire manufacturing workers (McMichael *et al.*
15 1976) and statistically nonsignificant increases were observed for combined
16 lymphohematopoietic cancers and some specific lymphohematopoietic cancers in the
17 Meinhardt and Matanoski cohorts, but the potentially confounding effects of butadiene
18 and other exposures were not analyzed. Two nested case-control studies (using different
19 types of analyses and exposure assessments and the same group of cases) from the
20 Matanoski cohort attempted to evaluate the relative contribution of styrene and butadiene
21 to lymphohematopoietic cancer mortality. Santos-Burgoa *et al.* (1992) found no
22 significant excess risks for combined and specific lymphohematopoietic cancers and
23 mean exposure after controlling for butadiene exposure. Matanoski *et al.* (1997)
24 calculated risks for both average and cumulative exposure to styrene. Taking into account
25 butadiene exposure, and demographic and employment variables in step-down regression
26 analyses, these models found, for an average exposure of 1 ppm vs. no exposure,
27 significant associations for all lymphohematopoietic cancers combined, lymphomas, and
28 myeloma, but not leukemia. For cumulative exposure, significant positive associations
29 between styrene exposure and combined lymphohematopoietic cancers, leukemia, and

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

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

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

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

1 all lymphohematopoietic (52 observed vs. 52.8 expected), lymphomas (14 vs. 15.1), or
2 leukemia (19 vs. 19.8), and were slightly higher than expected for Hodgkin's disease (11
3 observed vs. 7.9 expected) and multiple myeloma (4 vs. 3.4). Significantly increased
4 risks for leukemia incidence were reported in the Danish study among workers with
5 earlier first date of exposure, and who had worked at least 10 years since first
6 employment, but not for workers employed for 1 year or more (Kolstad *et al.* 1994). In
7 the European multi-country cohort (which overlaps with the Danish study), no excess of
8 leukemia mortality was found, and no exposure-response relationships with cumulative
9 or average exposure were observed, although a non-significant trend was observed with
10 time since first exposure (Kogevinas *et al.* 1994a). With respect to other
11 lymphohematopoietic cancers, non-significantly increased risks for non-Hodgkin's
12 lymphoma were found in the Danish and European multi-country cohorts. Positive
13 exposure-response relationships with average styrene exposure and time since first
14 exposure was observed for lymphohematopoietic cancers ($P = 0.019$ and 0.012 ,
15 respectively) and for malignant lymphoma ($P = 0.052$ and 0.072 , respectively) in the
16 European multi-country cohort, but no relationship with cumulative exposure was
17 observed (Kogevinas *et al.* 1994a). No excesses in mortality from any
18 lymphohematopoietic cancers were observed in the two smaller cohort studies (Ruder *et*
19 *al.* 2004 and Wong *et al.* 1994). In the styrene monomer and polymer industries, the risk
20 of lymphohematopoietic malignancies was also increased in most of the studies (as well
21 as the total number of observed cases across studies), but these workers might also have
22 been exposed to benzene.

23 *Pancreatic cancer*

24 Among the highest styrene-exposed group in the reinforced-plastics industry, there was
25 an excess in the total number of observed cases of pancreatic cancer across the four
26 cohort studies compared with the total number of expected cases [corresponding to an
27 SMR of 1.77 (95 % CI = 1.23 to 2.47)]. Increases in pancreatic cancer risk were observed
28 in three of the four reinforced-plastics industry cohorts (one of which was statistically
29 significant [Kolstad *et al.* 1995], and the other two of which were nonsignificant
30 [Kogevinas *et al.* 1994a, Ruder *et al.* 2004]). The risk of pancreatic cancer was slightly

1 higher among the Danish workers with longer term employment and earlier start date,
2 and increased with cumulative exposure in the multi-plant cohort. No indications of
3 exposure-response relationships were found in the smaller U.S. cohorts. Statistically
4 nonsignificant increased risks were also observed in one study in the styrene monomer
5 and polymer industry (Frentzel-Beyme *et al.* 1978), and among biomonitored workers
6 (10 years after the first measurement) (Anttila *et al.* 1998). However, no increased risk of
7 pancreatic cancer was reported among styrene-butadiene workers (Sathiakumar *et al.*
8 2005).

9 *Esophageal cancer*

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

21 *Other sites*

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

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

3 **Studies in Experimental Animals**

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

8 *Mice*

9 Three strains of mice were exposed to styrene by gavage. In male B6C3F₁ mice, exposure
10 to styrene for 5 days per week for 78 weeks was associated with a significantly increased
11 incidence of alveolar/bronchiolar adenoma and carcinoma (combined) in high-dose (300
12 mg/kg) animals, and a significant positive dose-response trend was observed (NCI
13 1979a). NCI questioned the significance of these lung tumors because the incidence in
14 the control group was unusually low compared with historical untreated controls, and
15 only small numbers of vehicle historical controls were available from the same testing
16 laboratory. [However, a larger number of vehicle (corn oil)-treated historical controls
17 from this same time period (prior to 1979), with similar study duration, and from the
18 same source as the styrene study were available from a different testing laboratory.
19 Results from these historical vehicle controls indicated that the concurrent vehicle
20 controls in the NCI study were not unusually low and the lung tumor incidence in the
21 high-dose group was significantly increased compared with those historical controls.]
22 There also was a significant dose-response trend for hepatocellular adenomas in female
23 B6C3F₁ mice, but no significant pair-wise comparisons were observed. The other gavage
24 study included a single dose of styrene administered to pregnant dams on gestation day
25 17 and weekly exposures of the pups after weaning (Pomomarkov and Tomatis 1978).
26 O20 mice (a strain with a high spontaneous incidence of lung tumors) were dosed at
27 1,350 mg/kg and C57Bl mice were dosed at 300 mg/kg. A significantly higher incidence
28 and earlier onset of lung tumors (adenoma and carcinoma combined) occurred in both
29 male and female O20 mice compared with vehicle controls. Tumor incidence was not
30 significantly increased in C57Bl mice.

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

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

11 *Rats*

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

17 An oral gavage study in F344 rats (NCI 1979a) and an inhalation study in Sprague-
18 Dawley rats (Cruzan *et al.* 1998) were the most robust and most completely reported
19 carcinogenicity studies. Neither study showed an increase in tumor incidences in styrene-
20 exposed rats, although Sprague-Dawley rats exhibited a negative trend in pituitary and
21 mammary gland tumors and a positive trend for testicular interstitial-cell tumors. In
22 another inhalation study in Sprague-Dawley rats, there was a dose-related increase in the
23 incidences of malignant mammary gland tumors; treatment-related and statistically
24 significant incidences of these tumors were seen in the top three dose groups (Conti *et al.*
25 1988). A drinking-water study did not report any dose-related carcinogenic effects
26 (Beliles *et al.* 1985). However, statistical reanalyses of study data indicated a marginal
27 increase in the incidence of mammary fibroadenoma in high-dose female rats and a
28 significant dose-related trend. Another inhalation study (Jersey *et al.* 1978) [unpublished
29 but reviewed in several published reports] indicated that styrene was associated with a

1 statistically significant increase in incidence of mammary adenocarcinoma in the low-
2 (600-ppm) but not high-dose (1000-ppm) group and a significant increase (when
3 compared with historical but not concurrent controls) in the combined incidence of
4 lymphosarcoma and leukemia in female rats in both the 600-ppm and 1000-ppm dose
5 groups. The authors did not consider the mammary adenocarcinomas to be causally
6 associated with styrene exposure because the incidence of mammary adenocarcinoma
7 was low compared with historical controls and there was no incidence of mammary
8 adenocarcinoma in the high-dose group. Elevated incidences of leukemia/lymphosarcoma
9 were observed in both treatment groups of female Sprague Dawley rats in this inhalation
10 study.

11 *Mixtures and Metabolite Studies*

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

18 The styrene metabolite, styrene-7,8-oxide, was previously evaluated for carcinogenicity
19 and is listed in the Report on Carcinogens [first listed in the 10th Report on Carcinogens,
20 2002] as *reasonably anticipated to be a human carcinogen* based on forestomach tumors
21 in rats and mice and liver tumors in male mice.

22 **Absorption, Distribution, Metabolism, and Excretion**

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

1 epoxide hydrolase. Styrene is metabolized in both the liver and the lung, and the Clara
2 cells in the lung are regarded as the major cell type in styrene activation following
3 inhalation exposure. The initial step in styrene metabolism is catalyzed by cytochromes
4 P450; CYP2E1 and Cyp2f2 are the predominant enzymes in humans and experimental
5 animals. In animals, CYP2E1 predominates in liver, while Cyp2f2 is the primary enzyme
6 in mouse lung. CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1 are preferentially
7 expressed in the lung compared with liver in humans, and the human CYP2F1 has been
8 shown to be capable of metabolizing styrene when expressed *in vitro*. Because styrene-
9 7,8-oxide contains a chiral carbon, this and some subsequent styrene metabolites can
10 exist as either *R*- or *S*-enantiomers. A second metabolic pathway through styrene-3,4-
11 oxide results in formation of 4-vinylphenol, which has been detected in humans, rats, and
12 mice *in vivo*, but the importance of 4-vinylphenol in styrene toxicity has not been well
13 characterized. Almost all absorbed styrene is excreted as urinary metabolites, primarily
14 mandelic acid and phenylglyoxylic acid.

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

23 **Toxicity**

24 Styrene exposure has been associated with numerous health effects in humans and
25 laboratory animals. The acute toxicity of styrene is low to moderate with an oral LD₅₀ of
26 320 mg/kg and an inhalation LC₅₀ of 4,940 ppm (4-hour exposure) in mice and an oral
27 LD₅₀ of 5,000 mg/kg and an inhalation LC₅₀ of 2,770 ppm (2-hour exposure) in rats. The
28 primary effects of acute exposure to styrene in experimental animals and humans include
29 irritation of the skin, eyes, and respiratory tract and CNS effects. Drowsiness, listlessness,

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

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

22 **Genetic Damage**

23 *In vitro* studies show that styrene-7,8-oxide forms DNA adducts and causes single-strand
24 breaks in a dose-related manner. Several studies have shown a correlation between
25 single-strand breaks and DNA adducts and indicate that the strand breaks, which are not
26 generally regarded as significantly lethal or mutagenic lesions, are efficiently repaired
27 within several hours after exposure has stopped. Adducts are formed primarily at the N7-,
28 N²-, and O⁶-positions of guanine. N7-adducts are formed in the greatest amount but are
29 the least persistent, while O⁶-adducts are formed in the least amount but are the most
30 persistent. Styrene-7,8-oxide was mutagenic without metabolic activation in all *in vitro*

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

9 DNA adducts, primarily N7- and O⁶-adducts, were reported in white blood cells in all
10 studies of styrene-exposed workers employed mainly in hand-lamination plants. In most
11 studies in workers, single-strand breaks showed exposure-related increases; however, two
12 studies gave negative results. The limited data on mutation frequencies in *HPRT* and
13 *GPA* in styrene-exposed workers are inconclusive. More than half the studies measuring
14 chromosomal aberrations have reported an increase in chromosomal aberrations in
15 styrene-exposed workers (or subgroups of workers), and several studies have reported a
16 positive exposure-response relationship with styrene air levels or urinary metabolites. A
17 meta-analysis of 22 studies found a positive association between styrene exposure level
18 and chromosomal aberration frequency when exposure levels were dichotomized as
19 greater than or less than a threshold value of 30 ppm for an 8-hour time-weighted
20 average. Studies of other cytogenetic markers in humans are conflicting. About half of
21 the studies that evaluated micronucleus and SCE frequency in styrene workers were
22 positive, and a few studies have reported significant dose-response relationships with
23 styrene exposure. A meta-analysis of 10 micronucleus studies was inconclusive, and a
24 meta-analysis of 14 SCE studies indicated a slight increase in SCE frequency but, again,
25 was too small to be conclusive. A number of studies have been published on the possible
26 modulating role of genetic polymorphisms, mainly in xenobiotic metabolism enzymes
27 and DNA-repair genes, at the level of various biomarkers. Some authors have suggested
28 that genetic susceptibility (probably at many loci) may be important in styrene-mediated
29 genotoxicity.

1 **Mechanistic Data**

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

17 Many researchers have tried to explain why lung tumors were observed in mice but not in
18 rats in long-term inhalation exposure studies. Some researchers have proposed that
19 styrene exposure causes pulmonary hyperplasia in the mouse lung, which may play a role
20 in the development of lung tumors. Effects of repeated styrene exposure observed in the
21 lungs of mice, but not in rats, included focal crowding of bronchiolar cells, bronchiolar
22 epithelial hyperplasia, and bronchiolo-alveolar hyperplasia. The Harvard Center for Risk
23 Analysis (Cohen *et al.* 2002) considered three factors as possible explanations for the
24 greater susceptibility of mouse lung than rat lung to development of hyperplasia leading
25 to tumors with exposure to styrene are: (1) the presence of the styrene-metabolizing
26 cytochromes in mouse lung tissues, (2) greater formation of the *R*-enantiomer of styrene-
27 7,8-oxide, and (3) the susceptibility of mouse lung tissue to glutathione depletion.
28 However, they concluded that although toxicokinetic models generally predict higher
29 rates of metabolism by mice and rats than by humans, the models do not consistently
30 predict a difference between the rodent species. An alternative mechanism is that

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

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Abbreviations

ABS:	acrylonitrile-butadiene-styrene
ACGIH:	American Conference of Governmental Industrial Hygienists
ADH:	alcohol dehydrogenase
ALDH:	aldehyde dehydrogenase
AIO:	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:	<i>Chemical Economics Handbook</i>
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 <i>in-situ</i> 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:	<i>N</i> -acetoxy-2-acetylaminofluorene
NAcT:	<i>N</i> -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:	<i>N</i> -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-(<i>N</i> -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
<i>r</i> :	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
XPB:	xeroderma pigmentosum, complementation group D
XPG:	xeroderma pigmentosum, complementation group G
XRCC:	X-ray repair cross-complementing group
yr:	year

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1 Introduction

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

7 Styrene was nominated for possible listing in the *Report on Carcinogens* by a private
8 individual based on its widespread use and exposure and evidence of carcinogenicity
9 from studies in humans and experimental animals. The International Agency for Research
10 on Cancer (IARC) currently classifies styrene as possibly carcinogenic to humans (Group
11 2B) based on limited evidence in humans and limited evidence in experimental animals
12 (IARC 1994a, 2002). Styrene-7,8-oxide, a major metabolite of styrene, has been
13 classified by the *Report on Carcinogens* as *reasonably anticipated to be a human*
14 *carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (NTP
15 2004). IARC (1994b) also classifies styrene-7,8-oxide as probably carcinogenic to
16 humans (Group 2A) based on sufficient evidence in experimental animals (forestomach
17 tumors in rats and mice and liver tumors in male mice) and mechanistic data.

18 1.1 Chemical identification

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

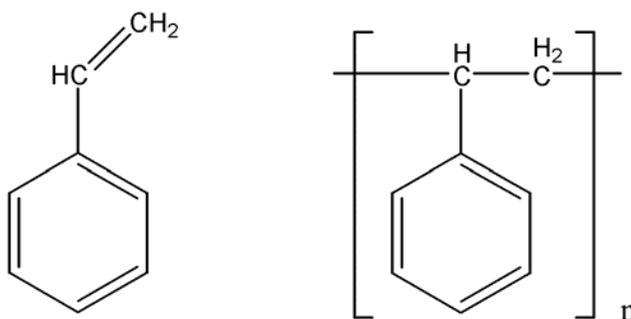


Figure 1-1. Chemical structures of styrene and polystyrene

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 ₈ H ₈	C ₈ H ₈ O
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 H₂O₂) (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, <i>n</i> -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 **1.3 Metabolites**

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

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

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

1 are nonsuperimposable mirror images): the *R*- and *S*-isomers (Figure 1-2). Epoxides are
2 oxygen-containing heterocyclic compounds that are highly reactive because of the strain
3 associated with the three-membered ring structure (Melnick 2002). The oxide forms are
4 further metabolized to styrene glycol (phenylethylene glycol) by microsomal epoxide
5 hydrolase. Styrene glycol is then oxidized by alcohol and aldehyde dehydrogenases to
6 form mandelic acid and phenylglyoxylic acid and their conjugates, the main urinary
7 metabolites. Known and hypothesized urinary styrene metabolites are shown in Table 1-
8 3.

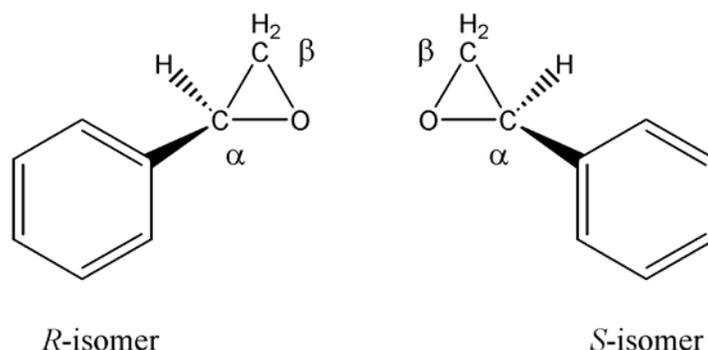
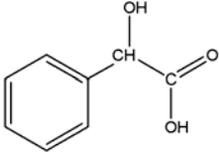
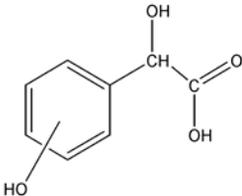
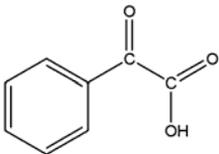
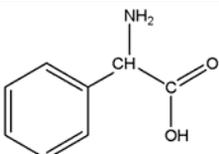
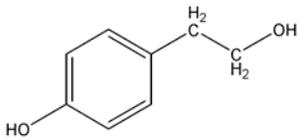
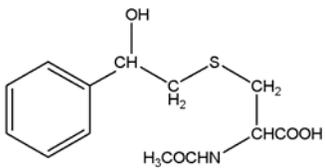
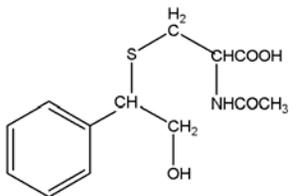
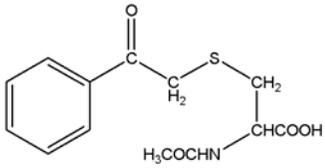
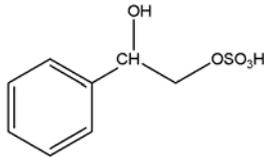
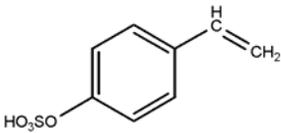
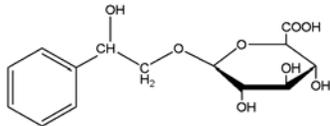
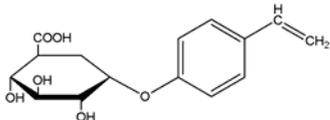


Figure 1-2. Stereoisomers of styrene-7,8-oxide (epoxyethylbenzene)

9 The stereoisomers of styrene-7,8-oxide are designated as *R*- or *S*- based on ordering the
10 molecule with the lowest priority atom (hydrogen) away from the viewer as designated
11 by the dashed line. The order of the remaining substituents from highest to lowest (i.e.,
12 oxygen, carbon in the benzene ring, carbon in CH₂) is oriented either clockwise (*R*-
13 isomer) or counterclockwise (*S*-isomer). Also, the two carbons attached to the benzene
14 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.

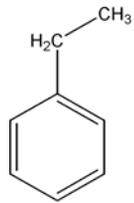
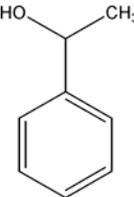
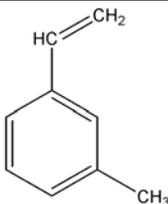
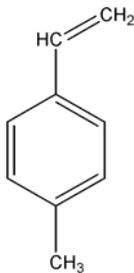
^aManini *et al.* (2002b) reported that this metabolite is expected to be formed, but it has never been demonstrated in urine following styrene exposure.

^bTwo diastereoisomers of each PHEMA exist (see Figure 5-1).

1 1.4 Analogues

2 Many chemical analogues of styrene exist. Table 1-4 shows the structures of four
3 analogues that are discussed in Section 5. Ethyl benzene is a chemical precursor for
4 styrene (see Section 2), and it and 1-phenylethanol are metabolites of styrene (see Figure
5 5-1). 3-Methylstyrene and 4-methylstyrene are often used as a mixture called
6 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.

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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,

1 until World War II when the United States initiated a major program to develop synthetic
 2 rubber (Miller *et al.* 1994, Steele *et al.* 1994). Since that time, styrene has become an
 3 important chemical used in the synthesis and manufacture of polystyrene and hundreds of
 4 different copolymers, as well as numerous other industrial resins (Guest 1997). Styrene
 5 producers sell styrene monomer to companies (resin manufacturers and compound
 6 producers) who use the styrene to make resins. Fabricators then process the resins into a
 7 wide variety of products (Cohen *et al.* 2002). Roughly 99% of the industrial resins
 8 produced from styrene can be grouped into six major categories. These six categories of
 9 resins (including unsaturated polyester resins with and without reinforcement), and some
 10 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.

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

1 electrical devices, in the production of automobile body parts, corrosion-resistant tanks
2 and pipes, in various construction items, carpet backings, house paints, paper processing,
3 computer printer cartridges, insulation products, wood floor waxes and polishes,
4 adhesives, putties, personal care products, and other items (IARC 2002, Luderer *et al.*
5 2005, NLM 2008).

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

12 **2.2 Production**

13 There are two commercially viable methods to produce styrene (ATSDR 1992, HSDB
14 2008a). The most common process, which accounts for over 90% of the total world
15 styrene production, involves catalytic dehydrogenation of ethylbenzene. In the Dow
16 Process, superheated steam is injected with ethylbenzene over a fixed catalytic reactor.
17 The catalyst is iron-oxide based and contains Cr_2O_3 and KOH or K_2CO_3 as promoters
18 (Cheresources 2008b). Ethylbenzene conversion is typically 60% to 65%, and there are
19 three significant byproducts: toluene, benzene, and hydrogen. After the reaction, the
20 products are cooled and the product stream, which contains styrene, toluene, benzene,
21 and unreacted ethylbenzene, is fractionally condensed. After adding a polymerization
22 inhibitor, the styrene is vacuum distilled to reach the required purity (noted as 99.8%).
23 The second process involves oxidation of ethylbenzene to its peroxide, which is then
24 reacted with propylene to produce propylene oxide and alpha-methylphenyl carbinol. The
25 carbinol is then dehydrated to produce styrene.

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

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

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

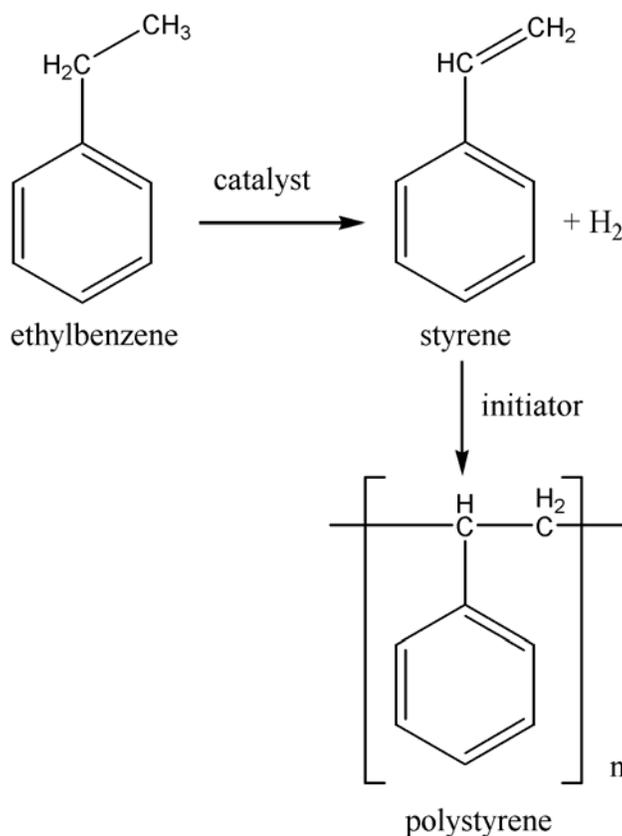


Figure 2-1. Synthesis of styrene from ethylbenzene and polymerization of styrene to form polystyrene

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

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

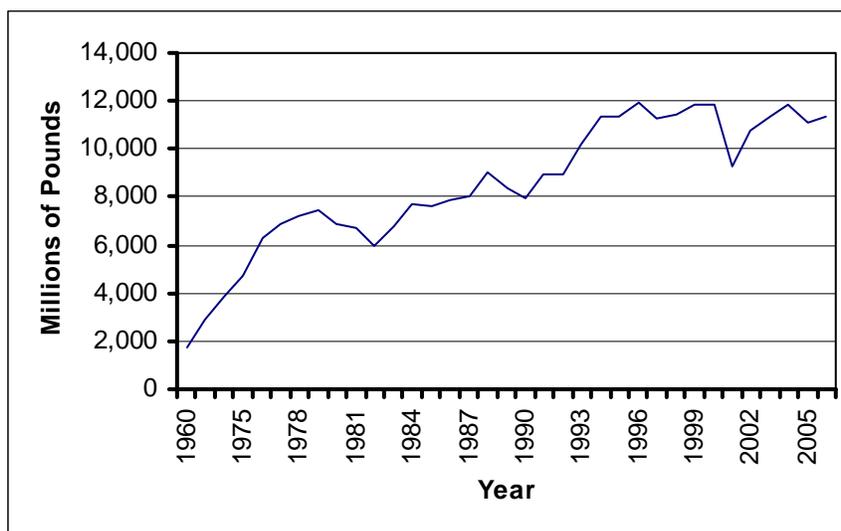


Figure 2-2. U.S. styrene production (1960–2006)

Source: (Berthiaume and Ring 2006).

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

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

14 During the 1990s, styrene consumption in the United States increased at an average
15 annual rate of 2.2% with over 99% consumed in the production of polymers and
16 copolymers (Berthiaume and Ring 2006). U.S. consumption of styrene in 2006 was 9,600
17 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.

1 **2.3 Environmental release, fate, and occurrence**

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

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

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

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

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

1 2.3.1 Air

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

9 2.3.1.1 Outdoor release

10 Major sources of styrene in outdoor air include industrial sources, automobile emissions,
11 and combustion processes such as waste incineration and the burning of wood (ATSDR
12 1992, IARC 1994a). Typical sources of industrial styrene emissions include facilities
13 producing styrene, polystyrene, other plastics, glass fiber–reinforced plastic products,
14 synthetic rubber, and resins (ATSDR 1992). For 2006 [the most recent data available],
15 the U.S. EPA’s Toxics Release Inventory (TRI) reported styrene fugitive air emissions of
16 9.9 million pounds, and point-source air emissions of 37.4 million pounds (TRI 2008a).
17 These air emissions combined [47.2 million pounds] accounted for roughly 93% of the
18 total TRI styrene releases for all reported environmental media in 2005. Between 1988
19 (the first year of TRI reporting) and 2005, the smallest reported total air release (point-
20 source plus fugitive emissions) was 30.3 million pounds in 1991 and the largest was 59.5
21 million pounds in 1999. Among the 519 TRI 2001 Core Chemicals, styrene had the 6th
22 highest level of point-source air emissions and the 5th highest level of fugitive air
23 emissions in 2005 (TRI 2008b, 2008c). [Note that since EPA’s reporting requirements are
24 for those facilities that produce or use large amounts of a chemical, actual emissions
25 probably are greater than those reported.]

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

1 2010 are expected to fall to 7,652 tons [15.3 million pounds] for highway vehicles and
2 2,297 tons [4.6 million pounds] for non-road equipment primarily as a result of
3 reductions due to the Mobile Source Air Toxics rule (see Section 2.7 for regulations and
4 guidelines).

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

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

17 2.3.1.2 *Indoor release*

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

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

- 1 Table 2-2 provides data on styrene monomer levels in various types of styrene polymer
- 2 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.

- 3 Historical levels of styrene from 1976 to 1980 are presented in Table 2-3. These data
- 4 show some reduction in residual styrene monomer levels from 1976 to 1980. Residual
- 5 levels have been further reduced since this time due to improvements in production
- 6 methods (EU 2002). Luderer *et al.* (2005) noted that the residual monomer data from
- 7 1980 may overestimate residual levels of styrene in polymers currently manufactured in
- 8 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.

^aTwo values were reported in original source without explanation.

^bIntended for food containers.

^cIntended for refrigerator applications.

^dIntended for household appliances.

1 There is limited information on losses of styrene to air from finished articles. EU (2002)
2 reported that based on an examination of three types of polystyrene to determine any loss
3 of residual monomer, no change was seen in flexible or rigid polystyrene cold drinks
4 cups over a six-month interval, although there appeared to be some loss (from 104 ppm to
5 71 ppm residual styrene) from foam hot drinks cups. Another study reviewed by the EU
6 reported that the residual monomer content of polystyrene and styrene copolymers (300
7 to 500 ppm) did not reveal any losses over 2 years. The EU also reported that typical
8 levels of styrene in expanded polystyrene molding will decrease from an initial level of
9 500 mg/kg to an equilibrium level of around 200 mg/kg over a period of 2 to 5 years
10 depending on use.

11 The European Union (EU 2002) reported that an emission factor of 0.03% [0.3 g/kg]
12 would be appropriate for long-term use (e.g., insulation in buildings) but would over-
13 estimate losses for short-term applications such as packaging. Rates of styrene emission
14 from glued carpet have been estimated at 98 ng/min per m² (ATSDR 1992). In a chamber
15 test of cork parquet flooring applied on concrete, styrene emissions were measured at 3

1 $\mu\text{g}/\text{h}\cdot\text{m}^2$ after 24 hours, 2 $\mu\text{g}/\text{h}\cdot\text{m}^2$ after 168 hours, and $< 1 \mu\text{g}/\text{h}\cdot\text{m}^2$ after 576 hours [the
2 source of the styrene was not specified] (Uhde and Salthammer 2007).

3 Infiltration of outdoor air has been proposed to be a potentially important source of
4 styrene levels in indoor air (Guo *et al.* 2004a). Infiltration of gasoline-related volatile
5 organic compounds (VOCs) from attached garages into houses is another potential source
6 of styrene in indoor air (Adgate *et al.* 2004b, Batterman *et al.* 2006, Batterman *et al.*
7 2007). In Minnesota residences, Adgate *et al.* found significantly higher levels of styrene
8 in homes with attached garages than in homes without attached garages. However, in
9 assessing styrene levels in houses and in garages of 15 Michigan residences with attached
10 garages, Batterman *et al.* found mixed results for styrene, with 8 of the 15 houses having
11 higher concentrations than the attached garages. The authors were unable to conclude that
12 styrene from the attached garages contributed to indoor air levels.

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

15 2.3.1.3 Fate

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

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

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

3 2.3.1.4 Outdoor occurrence

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

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

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

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

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

6 IARC (2002), Luderer *et al.* (2005), European Union (EU 2002), and HSDB (2008a)
 7 have presented results of monitoring studies from U.S. urban areas (Table 2-4). Payne-
 8 Sturges *et al.* (2004a) found that mean and median outdoor monitoring levels were less
 9 than indoor air monitoring levels and personal monitoring levels (see “Indoor
 10 Occurrence,” below); however, outdoor levels were higher than modeling results
 11 predicted by the U.S. EPA’s Assessment System for Population Exposure Nationwide
 12 (ASPEN) model. Similarly, Adgate *et al.* (2004a, 2004b) found higher styrene levels for
 13 indoor air monitoring and personal monitoring than for outdoor ambient air monitoring.
 14 For Table 2-4 and the remainder of the tables presenting environmental levels, the
 15 number of samples is presented when it was available in the referenced source; otherwise,
 16 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 Francisco, CA (NR)	range	9.83–36.73	HSDB 2008a
Pennsylvania turnpike tunnel (NR)	range	0.25–1.5	Luderer <i>et al.</i> 2005
Urban locations			
Baltimore, MD (2000–2001)	mean median	0.12 ^a 0.06 ^a	Payne-Sturges <i>et al.</i> 2004a
Minneapolis, MN (1997)	median, winter median, spring	0.023 ^a 0.0	Adgate <i>et al.</i> 2004a
Minneapolis, MN (2000)	mean	0.12 ^a	Adgate <i>et al.</i> 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 <i>et al.</i> 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 $\mu\text{g}/\text{m}^3$ in source document.

1 2.3.1.5 Indoor occurrence

2 Indoor air concentrations of styrene typically exceed outdoor air concentrations (Miller *et*
3 *al.* 1994). IARC (2002) reported that median residential air concentrations collected by
4 personal sampling have generally ranged from 1 to 3 $\mu\text{g}/\text{m}^3$ [0.2 to 0.7 ppb]. ATSDR
5 (1992) noted that mean indoor air levels of styrene have been reported in the range of 0.1
6 to 9 $\mu\text{g}/\text{m}^3$ [0.02 to 2.1 ppb] and can be attributed to off-gassing from building materials
7 and consumer products and from tobacco smoke. Fishbein (1992) reported typical indoor
8 levels ranging from 0.3 to 50 $\mu\text{g}/\text{m}^3$ [0.07 to 11.7 ppb]. Based on a U.S. EPA national
9 VOCs database compiled in the early- to mid-1980s from various sources of U.S. indoor
10 air concentration data, Miller *et al.* (1994) reported a mean indoor styrene air level of
11 1.413 ppb and a median level of 0.305 ppb based on 2,125 data points. Styrene levels
12 measured in indoor air in the United States are presented in Table 2-5.

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

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

1 concentrations for styrene of 0.12 $\mu\text{g}/\text{m}^3$ [0.03 ppb]; however, mean monitoring values
2 were 2.72 $\mu\text{g}/\text{m}^3$ [0.64 ppb] for indoor air, and 2.51 $\mu\text{g}/\text{m}^3$ [0.59 ppb] for personal
3 monitoring. The authors noted that indoor exposures were the dominant source of styrene
4 exposure.

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

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

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

3 In a study assessing VOC exposures to children in Minneapolis, MN, Adgate *et al.*
 4 (2004a) noted that styrene levels were more frequently detectable in home and personal
 5 samples than in outdoor samples or samples taken in the children’s schools. The authors
 6 reported slightly higher styrene air levels for home monitoring than for personal
 7 monitoring, and an 8-fold to almost 10-fold increase in home levels compared with
 8 school levels. In another study of VOC exposures in households with children (N = 284)
 9 in Minneapolis, MN, households with smokers, households with attached garages, and
 10 non-urban residences (which had a greater prevalence of smokers and attached garages)
 11 all had significantly higher levels of styrene (Adgate *et al.* 2004b).

12 ATSDR measures indoor air concentrations as part of their hazardous waste site
 13 investigations (HazDat 2008). Between 1980 and 2005, maximum concentrations
 14 measured in buildings onsite at hazardous waste sites were much higher than maximum
 15 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	1.413	Miller <i>et al.</i> 1994
	median	0.305	
Studies assessing microenvironments			
Minneapolis, MN (2000)	<i>School monitoring</i>		Adgate <i>et al.</i> 2004a
	winter (median)	0.02 ^a (N = 39)	
	spring (median)	0.02 ^a (N = 47)	
	<i>Home monitoring</i>		
	winter (median)	0.16 ^a (N = 93)	
	spring (median)	0.19 ^a (N = 88)	
	<i>Personal monitoring</i>		
	winter (median)	0.12 ^a (N = 93)	
	spring (median)	0.12 ^a (N = 88)	
Minneapolis, MN (1997)	<i>Screening assessment</i>		Adgate <i>et al.</i> 2004b
	indoor monitoring (mean)	0.28 ^a (N = 284)	
	<i>Intensive-phase assessment</i>		
	personal monitoring (mean)	0.28 ^a (N = 73)	

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 <i>et al.</i> 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 <i>et al.</i> 2007
Detroit, MI commuting routes (1999)	<i>Pilot study</i> range <i>Three day study</i> mean and range	0.07–0.26 ^a (N = 16) 0.26 ^a (0.02–0.82) (N = 48)	Batterman <i>et al.</i> 2002
Boston, MA (2003–2004)	stores (geometric mean) restaurants (geometric mean)	0.71 ^a (N = 89) 0.28 ^a (N = 20)	Loh <i>et al.</i> 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 $\mu\text{g}/\text{m}^3$ in source document.

^b Estimated from graph.

1 2.3.2 Water

2 2.3.2.1 Release

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

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

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

3 2.3.2.2 *Fate*

4 Volatilization and biodegradation are expected to be the major fate and transformation
5 processes in water. Based on its Henry's law constant, styrene is expected to volatilize
6 rapidly from environmental waters: the extent of volatilization depends on water depth
7 and turbulence with no volatilization occurring in stagnant deep water (ATSDR 1992,
8 Luderer *et al.* 2005). The estimated volatilization half-life of styrene from a river 1 m
9 deep with a current speed of 1 m/s and a wind velocity of 3 m/s is roughly 3 hours. Half-
10 lives have been estimated from 1 hour in a shallow body of water to 13 days in a lake,
11 and from 4 to 30 weeks in groundwater (Luderer *et al.* 2005). Some biological oxygen
12 demand studies have shown styrene to be biodegradable. Hydrolysis is not expected to be
13 an important degradation process. Adsorption to particulate matter and sediment may
14 have some significance, based on an organic carbon adsorption coefficient (K_{oc}) of 270 to
15 550 (Howard 1989). Styrene generally does not persist in water, because of its
16 biodegradability and volatility (Cohen *et al.* 2002).

17 2.3.2.3 *Occurrence*

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

23 *Drinking water*

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

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

4 *Environmental water*

5 Styrene has been found in the lower Tennessee River, the Kanawha River in West
 6 Virginia, the Great Lakes, and detected but not quantified in the Delaware River (EU
 7 2002, Howard 1989)

8 *Hazardous waste sites*

9 In the ATSDR *Toxicological Profile for Styrene*, it was reported that the geometric mean
 10 levels of styrene at U.S. hazardous waste sites were 9.3 µg/L [9.3 ppb] for surface water
 11 and 5.3 µg/L [5.3 ppb] for groundwater (ATSDR 1992). Styrene has been measured as
 12 part of ATSDR’s hazardous waste site investigations, which includes monitoring of on-
 13 site and off-site groundwater, on-site and off-site surface waters, and on-site tap water
 14 (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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1994
Groundwater, unspecified locations (NR)	945 groundwater samples in a drinking-water survey	ND	Miller <i>et al.</i> 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.

1 **2.3.3 Soil**

2 **2.3.3.1 Release**

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

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

5 2.3.3.2 *Fate*

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

17 2.3.3.3 *Occurrence*

18 There are limited data on styrene levels in soil. ATSDR has measured sediment and soil
19 concentrations of styrene as part of numerous hazardous waste-site investigations
20 (HazDat 2008). The maximum concentrations measured in sediment were 70 ppm on-site
21 and 0.37 ppm off-site. Soil concentrations were obtained at differing soil depths both on-
22 site and off-site. For samples taken from the top three inches of soil, concentrations on-
23 site were up to 14,000 ppm and off-site concentrations were up to 0.14 ppm. Surface top-
24 soil concentrations on-site were measured at levels up to 2,900 ppm. Subsurface soil
25 (deeper than 3 inches) was only measured on-site with a maximum concentration of
26 4,600 ppm. Because styrene is volatile, it is also present in soil gas and was measured
27 during the waste-site investigations both on-site and off-site. On-site soil gas
28 concentrations were up to 8,082,000 $\mu\text{g}/\text{m}^3$ [1,896 ppm], and off-site concentrations were
29 up to 690 ppb [0.69 ppm]. Sediment from the lower Tennessee River contained styrene at
30 4.2 ppb [0.0042 ppm] (Howard 1989).

1 2.3.4 Food

2 2.3.4.1 Sources of styrene in food

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

18 ATSDR (1992) reported that the rate at which styrene migrates from polystyrene
19 containers into food is mainly a function of the diffusion coefficient of the monomer in
20 the polymer and of the lipophilicity of the food. For example, 4% to 6% of the free
21 monomer in polystyrene packaging migrated into corn oil or sunflower oil within 10
22 days, while only 0.3% to 0.6% migrated into milk, beef, or water. Stoffers *et al.* (2004)
23 found the mean styrene migration rate from polystyrene into olive oil stored at 40°C for
24 10 days to be 0.013 mg/dm² [130 ng/cm²]. The authors noted that the migration was quite
25 low: only 1.8% of the initial styrene migrated. ATSDR (1992) reported styrene migration
26 from foam cups into liquids such as water, tea, or coffee to be about 8 ng/cm², while
27 migration into 8% ethanol, as might be encountered in wine or other alcoholic drinks,
28 was 36 ng/cm².

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

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

4 Temperature also has an impact on styrene migration. Lickly *et al.* (1995a) reported that
5 the log of the mean diffusion coefficient was linearly related to the inverse of the absolute
6 temperature of exposure from 70°F to 150°F [21°C to 66°C]. The mean diffusion
7 coefficients ranged from 4.5×10^{-11} cm²/s at 70°F to 3.4×10^{-9} cm²/s at 150°F. Choi *et al.*
8 (2005) examined the migration behavior of styrene monomer and oligomers from
9 polystyrene to the food simulants water and heptane, which are used to simulate aqueous
10 and fatty foods, respectively. Higher temperatures yielded faster migration rates, and the
11 higher molecular weight oligomers had slower migration rates than the styrene monomer.
12 Styrene can migrate into food from plastic containers during heating or cooking in
13 microwave or conventional ovens. Nerín and Acosta (2002) estimated the migration of
14 styrene and several other VOCs into food from five commercially available types of
15 plastic containers: polycarbonate, polypropylene copolymer, polypropylene random,
16 polypropylene-20% talcum, and styrene-acrylonitrile. Styrene migration was estimated at
17 levels ranging from 1.8×10^{-6} to 6.7×10^{-4} mg/kg of food. The experiment was conducted
18 in 120°C to 150°C [250°F to 300°F] ovens for 30 minutes. The maximum migration level
19 was from a styrene-acrylonitrile container, and the minimum migration level was from a
20 container made of polypropylene copolymer.

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

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

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

7 **2.3.4.2 Styrene levels in food**

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

16 Miller *et al.* (1994) and HSDB (2008a) summarized the results of several studies that
 17 measured styrene concentrations in foods packaged in polystyrene. Table 2-7 summarizes
 18 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).

^aA range is provided if the source document provided a range or if data are combined across sources.

- 1 Based on a literature review, Cohen *et al.* (2002) presented data on styrene levels
- 2 measured in various foods (Table 2-8). Styrene occurs naturally in some foods and
- 3 beverages (Miller *et al.* 1994, Steele *et al.* 1994, Tang *et al.* 2000), [and although it was
- 4 not specified whether levels presented by Cohen *et al.* were measured by a process that
- 5 avoided contact with styrene, it is likely that these data represent naturally occurring food
- 6 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.

- 1 Using a process that avoided contact with styrene or any type of plastic, Steele *et al.*
- 2 measured styrene concentrations in 12 types of raw agricultural products, with results
- 3 suggesting that styrene may be a natural constituent of many foods. Of 12 foods, 8 had
- 4 detectable styrene levels, from a low of 0.233 ng/g [ppb] for Oregon peaches to a high of
- 5 39,200 ng/g [ppb] for cinnamon from Indonesia. [It is noteworthy that three cinnamon
- 6 samples from three different sources were analyzed, and concentrations ranged from 179
- 7 ng/g to the high of 39,200 ng/g.] Other studies that have measured natural levels in foods
- 8 (*i.e.*, without contact with polystyrene) have yielded similar results (Miller *et al.* 1994).

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

5 Whole body concentrations of styrene ranging between 15 and 100 µg/kg have been
6 measured in splake and walleye fish caught in the St. Clair River, Canada. Styrene was
7 also detected, but not quantified, in several other fish from the St. Clair River (EU 2002).
8 Edible shellfish from Atlantic Canada were reported to contain styrene at levels less than
9 10.0 µg/kg. In a Japanese survey in 1986, styrene was found in 28 of 131 samples of fish
10 at concentrations ranging from 0.5 to 2.3 µg/kg (limit of detection 0.5 µg/kg).

11 Since 1991, the U.S. FDA has measured styrene in U.S. foods in its Total Diet Study
12 (TDS). The TDS measures levels of various contaminants and nutrients in foods that are
13 prepared as they would be consumed, so the results can be used to provide a realistic
14 measure of intake. Foods are purchased from supermarkets in selected U.S. cities,
15 generally three to four times per year, and shipped to a central FDA laboratory, where
16 they are prepared and analyzed (foods are measured as raw commodities if they are
17 generally consumed as such). Table 2-9 summarizes styrene levels detected in TDS food
18 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.

1 **2.4 General population exposure**

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

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

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

5 Exposure from ingestion of municipal drinking-water supplies probably is negligible, as
 6 styrene has been detected in drinking-water monitoring surveys infrequently, and when it
 7 has been detected, it has generally been at low levels. Ingestion of contaminated
 8 groundwater, however, could result in significant exposure (ATSDR 1992, Howard
 9 1989). Because of the low occurrence and levels of styrene in water, Cohen *et al.* (2002)
 10 suggested that dermal exposures from water could be assumed to be negligible.

11 Fishbein (1992) estimated the relative significance of different routes of exposure to
 12 styrene to illustrate the importance of both indoor air exposures and occupational
 13 exposures. The results of this analysis are presented in Table 2-10. These results show
 14 that occupational exposures result in the highest styrene intakes; however, the general
 15 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 µg/m³ in source document.

^b Based on the assumption of a daily breathing volume of 10 m³ at work or 20 m³ at home or in an urban environment.

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

1 indoor air concentration of 0.28 µg/m³ was used, which is similar to the low-end value
 2 used by Fishbein (1992) (above). Estimates for exposure from smoking assumed that
 3 styrene content in mainstream cigarette smoke is 10 µg/cigarette [which was half the
 4 level of the minimum of the range of values presented by Fishbein above], and that 20
 5 cigarettes per day are smoked. [Note that the exposure values in this table are presented
 6 in units of µg/kg b.w. and are not directly comparable to most of the data in this section,
 7 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.

8 Smoking can result in styrene exposure both directly for smokers and indirectly through
 9 environmental tobacco smoke (ETS) (*i.e.*, side-stream smoke and exhaled cigarette
 10 smoke). Exposure to styrene has been estimated to be six times higher for smokers than
 11 for nonsmokers (Cohen *et al.* 2002). As noted in Table 2-10, Fishbein (1992) estimated a
 12 styrene exposure of 400 to 960 µg/day based on 20 cigarettes per day and inhalation of
 13 20 to 48 µg of styrene per cigarette. Tang *et al.* (2000) estimated an additional styrene
 14 intake (above the daily intake from air and food) of 100 µg/day, based on 20 cigarettes
 15 per day and inhalation of 5 µg styrene per cigarette. In a study on toxic compounds in
 16 ETS, Bi *et al.* (2005) presented styrene levels in ETS for three types of cigarettes: ultra
 17 low tar (146 µg/cigarette), full flavor low tar (159 µg/cigarette), and full flavor (119

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

11 Exposure to styrene from food ingestion has been estimated in a number of studies.
12 Lickly *et al.* (1995b) estimated U.S. dietary styrene exposure at 9 µg/day. A study of
13 residents of the United Kingdom in 1983 showed styrene intake from food ingestion of 1
14 to 4 µg/day (Lickly *et al.* 1995b). Another study of U.K. residents, which employed a
15 probabilistic modeling approach, estimated median daily intake of styrene from food
16 contaminated with food contact materials to be 0.039 µg/kg b.w. per day for adults, 0.048
17 µg/kg b.w. per day for youths, and 0.035 µg/kg b.w. per day for seniors (Holmes *et al.*
18 2005). Another study, based on the average *per capita* consumption figures of the general
19 population in Germany, estimated the average annual styrene intake via food
20 consumption to be roughly 0.8 to 4.5 mg/person [2.2 to 12.3 µg/d] (Tang *et al.* 2000).
21 Using the same data and applying a U.S. FDA consumption factor based on the
22 assumption that only 10% of foods are packaged in polystyrene, an annual intake of 0.08
23 to 0.45 mg/person [0.22 to 1.23 µg/d] was estimated. Other studies have estimated annual
24 per-person styrene intake via food ingestion ranging from 0.26 to 14.8 mg [0.7 to 40.5
25 µg/d] (Tang *et al.* 2000).

26 In an exposure and risk assessment for styrene, Cohen *et al.* (2002) based their
27 assessment only on inhalation and food ingestion exposures, assuming that exposure from
28 ingestion and dermal contact with water is negligible due to its limited occurrence and
29 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.

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

10 Several studies have confirmed styrene exposure to the general public through the use of
 11 biological monitoring. In one study, styrene was detected in all eight human breast milk
 12 samples from women in four U.S. cities (Howard 1989). In a National Human Adipose
 13 Tissue Survey by the U.S. EPA in 1982, styrene was detected in wet adipose tissue with a
 14 frequency of 100% at concentrations ranging from 8 to 350 ppb. Styrene also has been
 15 detected in the general population in blood at a mean concentration of 0.4 µg/L and in
 16 exhaled breath at mean concentrations of 0.7 to 1.6 µg/m³ (ATSDR 1992).

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

1 Reference Range Study assessed blood levels of numerous VOCs among a nonstatistical
2 subsample of NHANES III participants aged 20 to 59 (NCHS 2000). Samples in which
3 styrene was below the detection limit were assigned a value of 0.013 $\mu\text{g/L}$, which is equal
4 to the lower detection limit (0.019 $\mu\text{g/L}$) divided by the square root of 2. Of 624 samples,
5 styrene levels were below the detection limit in 78 samples (12.5%), and ranged from
6 0.019 to 4.006 $\mu\text{g/L}$ in 546 samples. The mean styrene level for all 624 samples was 0.07
7 $\mu\text{g/L}$, the median was 0.04 $\mu\text{g/L}$, and the 95th percentile value was 0.18 $\mu\text{g/L}$ (Ashley *et*
8 *al.* 1994, Sexton *et al.* 2005). [The means obtained by assigning a value of 0.013 $\mu\text{g/L}$ to
9 samples below the detection limit or by assigning a value of 0.00 $\mu\text{g/L}$ to these samples
10 were the same after rounding.] It is important to note that because this study was
11 conducted with a nonstatistical subsample of NHANES III participants, statistical
12 weights cannot be assigned, and estimates for the total U.S. population therefore cannot
13 be calculated (NCHS 2000).

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

1 median for the group was 0.07 ng/mL ($\mu\text{g/L}$) and the 95th percentile was 0.11; levels that
2 the authors noted were similar to the NHANES levels.

3 **2.5 Occupational exposure**

4 In some workplace settings, styrene air levels can exceed by several orders of magnitude
5 the levels generally found in outdoor and indoor air. [Because of this, air levels in this
6 section are presented in parts per million [ppm] rather than parts per billion [ppb], which
7 were used in the outdoor air and indoor air sections above.] Workers in a number of
8 different industries can be exposed to styrene. Workers can be exposed during the
9 production and use of styrene monomer, polystyrene, glass fiber–reinforced plastics,
10 styrene-butadiene rubber and other styrene-based polymers, and in other miscellaneous
11 occupations (ATSDR 1992, IARC 2002). The National Occupational Hazard Survey,
12 conducted by the National Institute for Occupational Safety and Health (NIOSH) from
13 1972 to 1974, estimated that 292,018 employees were occupationally exposed to styrene
14 at 16,394 facilities. The National Occupational Exposure Survey, conducted by NIOSH
15 from 1981 to 1983, estimated that 333,212 employees (including 86,902 women) were
16 occupationally exposed to styrene at 24,702 facilities in 154 industries. The U.S. Bureau
17 of Labor Statistics (BLS) uses the Standard Occupational Classification (SOC) system to
18 classify workers into occupational categories for labor statistics analyses. Workers are
19 classified into one of over 820 occupations according to their occupational definitions. In
20 May 2006, the BLS estimated that 32,510 workers were employed in SOC code 51-
21 2091 — Fiberglass Laminators and Fabricators (defined as “laminated layers of fiberglass
22 on molds to form boat decks and hulls, bodies for golf carts, automobiles, or other
23 products”). “Ship and boat building” was the largest subcategory in this SOC segment,
24 with 12,910 employees (BLS 2007). No information was found on the numbers of
25 workers in the other industrial segments mentioned above.

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

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

4 *2.5.1 The reinforced plastics industry*

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

27 *2.5.1.1 Process description*

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

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

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

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

4 2.5.1.2 Historical industry-wide exposure levels

5 Historically, the highest styrene exposure levels for reinforced-plastics workers has been
6 in the range of several hundred parts per million, although declining levels have been
7 reported to have occurred over the past several decades. In a study published in 1981 of
8 12 plants manufacturing fiberglass in Washington state, 40% of 8-hour samples contained
9 styrene at over 100 ppm (IARC 2002). Kolstad *et al.* (1994) presented styrene exposure
10 level data based on 2,473 personal air samples taken at workplaces in Denmark between
11 1964 and 1988 (see Section 3.1.4). Mean styrene levels were 180 ppm for 1964 to 1970,
12 88 ppm for 1971 to 1975, and 43 ppm for 1976 to 1988. In an extension of the 1994
13 study, Kolstad *et al.* (2005) used data from 2,454 personal measurements of airborne
14 styrene taken by the Danish National Institute of Occupational Health between 1960 and
15 1996 to develop a semi-quantitative method to assess occupational exposure to styrene in
16 the reinforced plastics industry when individual data are not available. Calendar year was
17 reported to be a strong and consistent predictor of styrene exposure levels; along with
18 product produced (boats) and process (hand and spray lamination). For the time period
19 between 1960 and 1990, styrene exposure levels were reported to have declined by 7%
20 annually. Kolstad *et al.* calculated exposure scores for individuals based on estimated
21 exposure probability and exposure levels. Styrene exposure scores for 1,519 subjects
22 based on short-term and long-term samples are presented in Figures 2-4 and 2-5 below.
23 Exposure scores declined by about 10-fold from the 1960s to the 1990s, and the authors
24 noted that this reflected a decline in styrene exposure levels. This study did not assess
25 dermal exposures.

26 Similarly, Kogevinas *et al.* (1994a) reported that in Denmark, average exposure levels
27 among laminators were about 200 ppm in the late 1950s, about 100 ppm in the late
28 1960s, and about 20 ppm in the late 1980s (see Section 3.1.5). In a review of 16 studies
29 by Pfäffli and Säämänen (1993), a similar temporal decline in exposure levels was seen

1 in air concentration data from the United States, Canada, Japan, and Europe from the
 2 1950s through 1992.

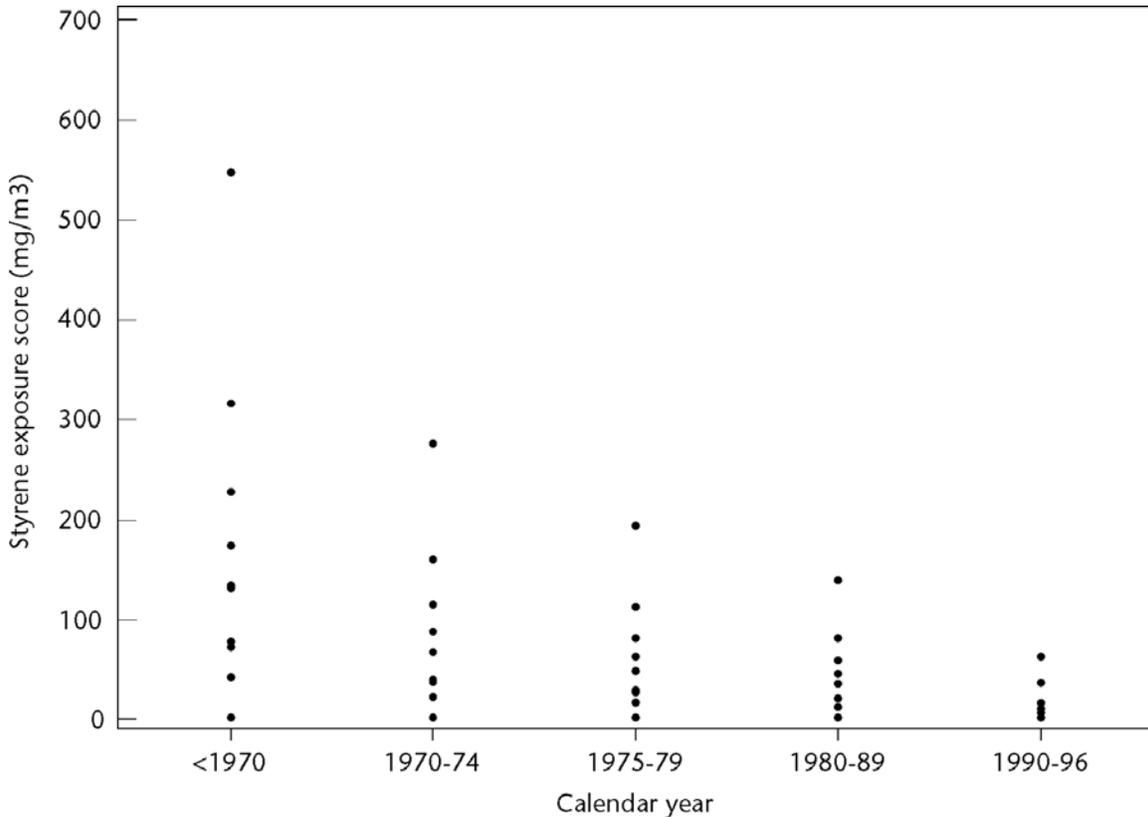


Figure 2-4. Temporal decline in styrene exposure scores (short-term samples [< 1 h]) estimated for reinforced plastics workers

Source: Kolstad *et al.* 2005.

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

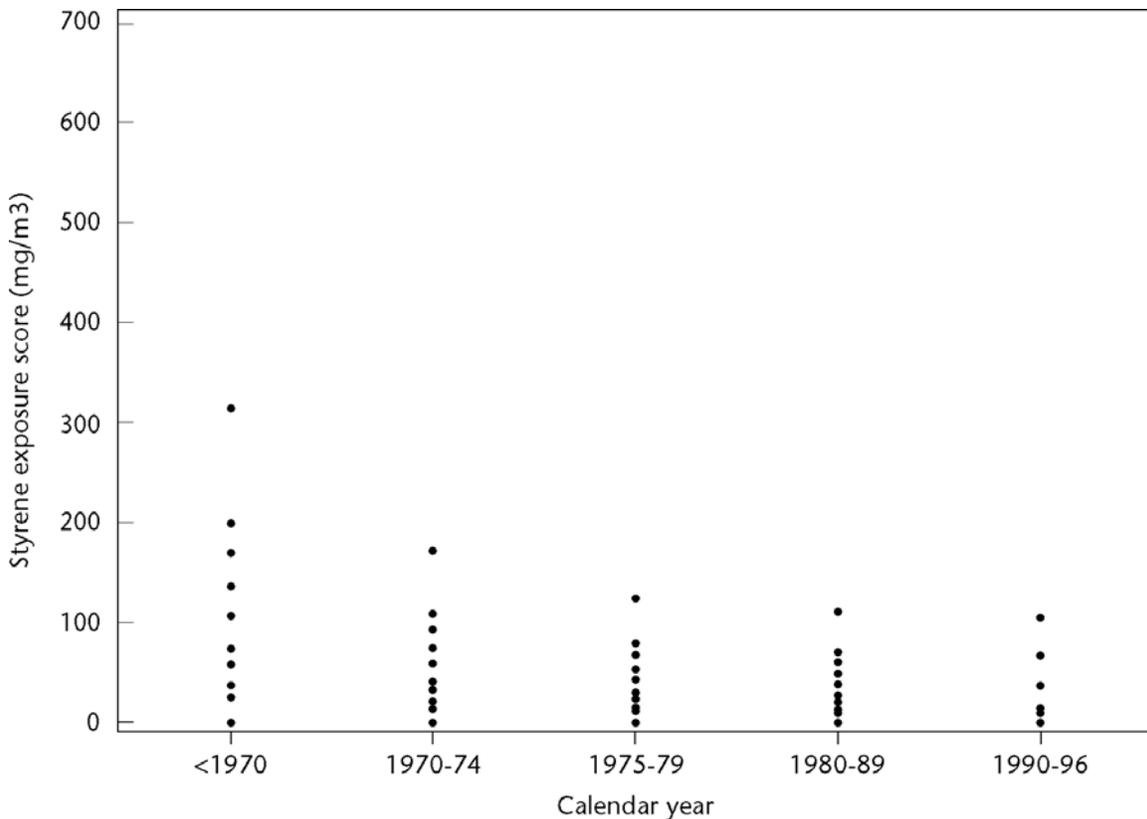


Figure 2-5. Temporal decline in styrene exposure scores short-term samples (< 1 h) estimated for reinforced plastics workers

Source: Kolstad *et al.* 2005.

1 Serdar *et al.* (2006) noted that in general, air levels of styrene (and styrene-7,8-oxide)
 2 appear to have decreased substantially in this industry from the 1980s through the early
 3 2000s. Although styrene exposures have been reduced substantially through improved
 4 work practices and products (Kolstad *et al.* 1994), Miller *et al.* noted in 1994 that peak
 5 concentrations could still exceed 100 ppm, especially during the manufacture of large
 6 items, and this can be seen in Table 2-13 for measurements taken through the 1990s.

7 2.5.1.3 Exposure levels based on product or manufacturing process

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

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

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

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

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

24 2.5.1.4 Exposure levels based on job or task

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

1 mg/g creatinine), rollers (327 mg/g creatinine), semiautomatic process operators (243
2 mg/g creatinine), and non-process workers (186 mg/g creatinine). Similarly, mean
3 styrene air levels were highest for hand laminators (227 mg/m³ [53 ppm]); however,
4 rollers were exposed to the next highest levels (163 mg/m³ [38 ppm]), followed by spray
5 laminators (134 mg/m³ [31 ppm]), and semiautomatic process operators (85 mg/m³ [20
6 ppm]). The authors noted a clear positive correlation between air levels and urinary
7 mandelic acid (MA) levels. The authors also noted that air levels generally decreased
8 with time.

9 Based on U.S. data published in 1985, IARC (2002) reported mean personal breathing-
10 zone air concentrations for four boat fabrication tasks as follows: hull lamination, 331
11 mg/m³ [77 ppm]; deck lamination, 313 mg/m³ [73 ppm]; small parts lamination, 193
12 mg/m³ [45 ppm]; and gel coating, 202 mg/m³ [47 ppm]. Measured concentrations across
13 all jobs or tasks ranged from 7 to 780 mg/m³ [1.6 to 183 ppm].

14 Based on results from one study that assessed 8-hour TWA exposure levels across job
15 categories and tasks within the reinforced plastics industry, spray-up/lay-up operators had
16 the highest exposure levels with a mean of 256 mg/m³ [60 ppm] and a range of 21 to 511
17 mg/m³ [5 to 120 ppm] (IARC 2002). For other jobs, which included gel coating and 7
18 other job categories, the mean exposure levels ranged from ≤ 43 to 192 mg/m³ [≤ 10 to
19 45 ppm], and overall exposure levels ranged from 0 to 362 mg/m³ (0 to 85 ppm). Based
20 on a study of 237 workers in 30 Finnish reinforced plastics plants, Nylander-French *et al.*
21 (1999) reported that the highest 8-hour TWA styrene exposure level across 6 categories
22 of tasks was for hand lamination of large objects (156 mg/m³ [37 ppm]) and that these
23 levels were approximately 4-fold higher than exposure levels for foremen (42.6 mg/m³
24 [10 ppm]), which was the group with the lowest exposure levels. Overall, the mean 8-
25 hour TWA concentration of styrene was 122 mg/m³ [28.6 ppm] with a range of 3.2 to 608
26 mg/m³ [0.75 to 142.7 ppm].

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

1 mg/m³ [33 ppm] while laminators had the second highest levels at 130 mg/m³ [30.5
2 ppm]. Overall, the mean styrene air level was 64.3 mg/m³ [15.1 ppm] with a range of
3 0.978 to 235 mg/m³ [0.23 to 55.14 ppm].

4 In Finnish factories that produced boats, car parts, and building materials from polyester-
5 based reinforced plastics, average styrene concentrations in personal air samples were
6 133 ppm for hand applicators and 130 ppm for spray applicators (IARC 1994b). Based on
7 2,528 measurements of styrene at 256 workplaces in Denmark between 1955 and 1988,
8 Jensen *et al.* reported that the work processes associated with the highest concentrations
9 were spray-up and unspecified lay-up operations.

10 Fairfax and Swearngin (2005) reported the results of a 2003 planned inspection by OSHA
11 of a facility that produced bathtubs and shower stalls by spray application of styrene-
12 based gel coat and polyester resin. The OSHA inspection consisted of full-shift personal
13 air monitoring of two gel-coat operators and two chopper gun operators and showed
14 TWA styrene levels ranging from 64 ppm (chopper gun operator) to 318 ppm (gel-coat
15 operator). Follow-up measurements (personal and area) were made in June and October
16 of 2003. June TWA levels ranged from 66 ppm to 100 ppm (both were gel-coat
17 operators), and October levels ranged from 45 ppm (gel-coat operator) to 110 ppm
18 (chopper gun operator). Ultimately, switching to a product containing less styrene and
19 operational changes were needed to reduce exposure levels below the regulatory limit of
20 100 ppm.

21 2.5.1.5 *Studies assessing respirator use*

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

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

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

15 In a study to assess the capacity of negative-pressure half-mask respirators to protect
16 workers from styrene exposure, personal sampling of styrene air levels and urinary
17 styrene levels was performed on seven fiberglass-reinforced plastics workers over two
18 successive weeks: one week without respirators and the following week with respirators
19 (Gobba *et al.* 2000). During the study period, mean TWA workplace air concentrations of
20 styrene were estimated for Monday, Wednesday, and Friday for both morning shifts and
21 afternoon shifts. These six mean TWA air concentrations ranged from 169.2 to 335.7
22 mg/m^3 [39.6 to 78.6 ppm] with a range across all measurements of 70.9 to 488.1 mg/m^3
23 [16.6 to 114.5 ppm]. Mean urinary styrene levels ranged from 80 to 96.1 $\mu\text{g}/\text{L}$ without
24 the use of respirators and from 31.5 to 47.2 $\mu\text{g}/\text{L}$ with the respirators. The estimated
25 reduction of urinary styrene levels due to the respirators ranged from 30% to 90% with a
26 mean of 60%.

27 2.5.1.6 Styrene-7,8-oxide exposures

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

1 substances showing styrene levels at two to three orders of magnitude higher than
2 styrene-7,8-oxide levels. Serdar *et al.* (2006) (discussed above) noted that styrene levels
3 in full-shift personal breathing-zone samples were roughly 500-fold higher than styrene-
4 7,8-oxide levels.

5 For 237 workers in 30 Finnish reinforced plastics plants, the mean 8-hour TWA
6 concentration of styrene was 122 mg/m³ [28.6 ppm] with a range of 3.2 to 608 mg/m³
7 [0.75 to 142.7 ppm], while the mean concentration of styrene-7,8-oxide was 0.183 mg/m³
8 [0.04 ppm] with a range of 0 to 0.883 mg/m³ [0 to 0.21 ppm] (Nylander-French *et al.*
9 1999). The authors found that styrene-7,8-oxide levels were positively correlated with
10 styrene exposure levels.

11 In a boat manufacturing factory in the United States, Rappaport *et al.* (1996) reported a
12 mean styrene air level of 64.3 mg/m³ [15.1 ppm] with a range of 0.978 to 235 mg/m³
13 [0.23 to 55.14 ppm] and a mean styrene-7,8-oxide level of 0.159 mg/m³ [0.037 ppm]
14 with a range 0.0134 to 0.525 mg/m³ [0.003 to 0.12 ppm]. IARC (1994b) reported that for
15 the 19 most heavily exposed workers in a boat manufacturing company, the mean styrene
16 exposure level was 64 mg/m³ [15 ppm] while the mean styrene-7,8-oxide level was 0.14
17 mg/m³ [0.03 ppm]. In Finnish factories that produced boats, car parts, and building
18 materials from polyester-based reinforced plastics, average styrene concentrations in
19 personal air samples were 133 ppm for hand applicators and 130 ppm for spray
20 applicators; the corresponding average styrene-7,8-oxide levels were 0.04 ppm and 0.12
21 ppm.

22 Table 2-13 presents styrene levels in ambient air in the reinforced plastics industry. For
23 this table and the remainder of the tables presenting occupational exposure levels, the
24 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)	<i>Tasks across all categories</i> <i>Production area</i> 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 <i>et al.</i> 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 <i>et al.</i> 2002 (Japan)
Industry wide (1960–1996)	<i>Overall short-term</i> ^b <i>Product</i> boats other products <i>Task</i> hand or spray lamination other <i>Year</i> 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 <i>et al.</i> 2005 (Denmark) [note that this study is an extension of Kolstad <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1985, Ruder <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2003, Lees <i>et al.</i> 2003 (USA)
Boats, tanks,	various processes and jobs	1.1–15.6 ^d (NR)	MA: 24.6–227 (NR) mg/g CR	Liljelind <i>et al.</i> 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)	<i>Overall</i> <i>Product specific mfg.</i> 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 ^c (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 <i>et al.</i> 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 <i>et al.</i> 1998 (Finland)
Boats, containers, pipes and tubes, small parts, sheets, and vehicle parts (1988–1990)	<i>Jobs</i> 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 <i>et al.</i> 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 <i>Products</i> 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 <i>et al.</i> 1994 (Denmark)
Industry-wide	overall TWA concentrations spray-up/lay-up operators TWA	1–200 60 ^c (5–120)	–	Wong <i>et al.</i> 1994 (USA)
Industry-wide (NR)	<i>Process workers</i> 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 <i>et al.</i> 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 <i>Non-process workers</i>	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 <i>et al.</i> 1990 (Denmark)
Industry-wide (1969–1981)	open-mold press-mold	3–82 (NR) (N = 1,084) 4–26 (NR) (N = 402)	–	Lemasters <i>et al.</i> 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.

^aMedian.

^bData also presented in source document for long-term samples showing levels that were generally around one-half the levels reported here.

^cPresented in mg/m³ in source document.

^dGeometric mean(s).

^eReported as typical level.

1 2.5.1.7 *Dermal exposure*

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

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

22 In an experiment to assess skin absorption of the hand and forearm of liquid styrene or
23 styrene in aqueous solution, Dutkiewicz and Tyras (1968) reported that very short
24 exposure of the hands to liquid styrene (a few minutes) or longer exposure (about one
25 hour) to styrene in aqueous solution can result in the absorption of as much styrene as an
26 8-hour average air concentration of 0.05 mg/L [50 mg/m³ or 11.7 ppm]. They also noted
27 that urinary mandelic acid does not provide a reliable index of absorption if there is
28 simultaneous skin and lung exposures.

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

1 upper back had the highest exposures. Potential total-body styrene exposure ranged from
2 544 to 17,100 mg/hour, with a geometric mean of 3,780 mg/hour. Wiczorek (1985)
3 investigated dermal absorption of styrene vapors in four volunteers exposed to styrene at
4 1,300 to 3,200 mg/m³ in a study chamber. The authors calculated that the dermal
5 absorption of styrene vapors contributed about 5% to the amount absorbed in the
6 respiratory tract under the same experimental conditions based on comparative mandelic
7 acid and phenylglyoxylic acid urinary levels. The authors presented a dermal vapor
8 absorption coefficient of 0.022 m³/hour based on the results of this study.

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

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

13 *2.5.2 The styrene-butadiene rubber (SBR) industry*

14 Styrene-butadiene rubber is a copolymer of butadiene and styrene in which the styrene
15 units (approximately 25%) are distributed at random among butadiene units (75%) in
16 molecular chains (IISRP 1973). Styrene-butadiene rubber is the most widely used
17 synthetic rubber in the world, accounting for 46% of world consumption of synthetic
18 rubber and more than 26% of all rubber, natural or synthetic, in 2006 (ICIS 2008). Over
19 70% of styrene-butadiene rubber is consumed in the manufacture of tires and tire
20 products; however, non-tire uses are growing with applications including conveyor belts,
21 gaskets, hoses, floor tiles, footwear, and adhesives. This section provides a brief
22 overview of the two main styrene-butadiene rubber production processes (emulsion
23 process [Section 2.5.2.1] and solution process [Section 2.5.2.2]), followed by a discussion
24 of exposure levels that have been found within the styrene-butadiene rubber industry
25 (Section 2.5.2.3).

26 *2.5.2.1 Emulsion process styrene-butadiene rubber production*

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

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

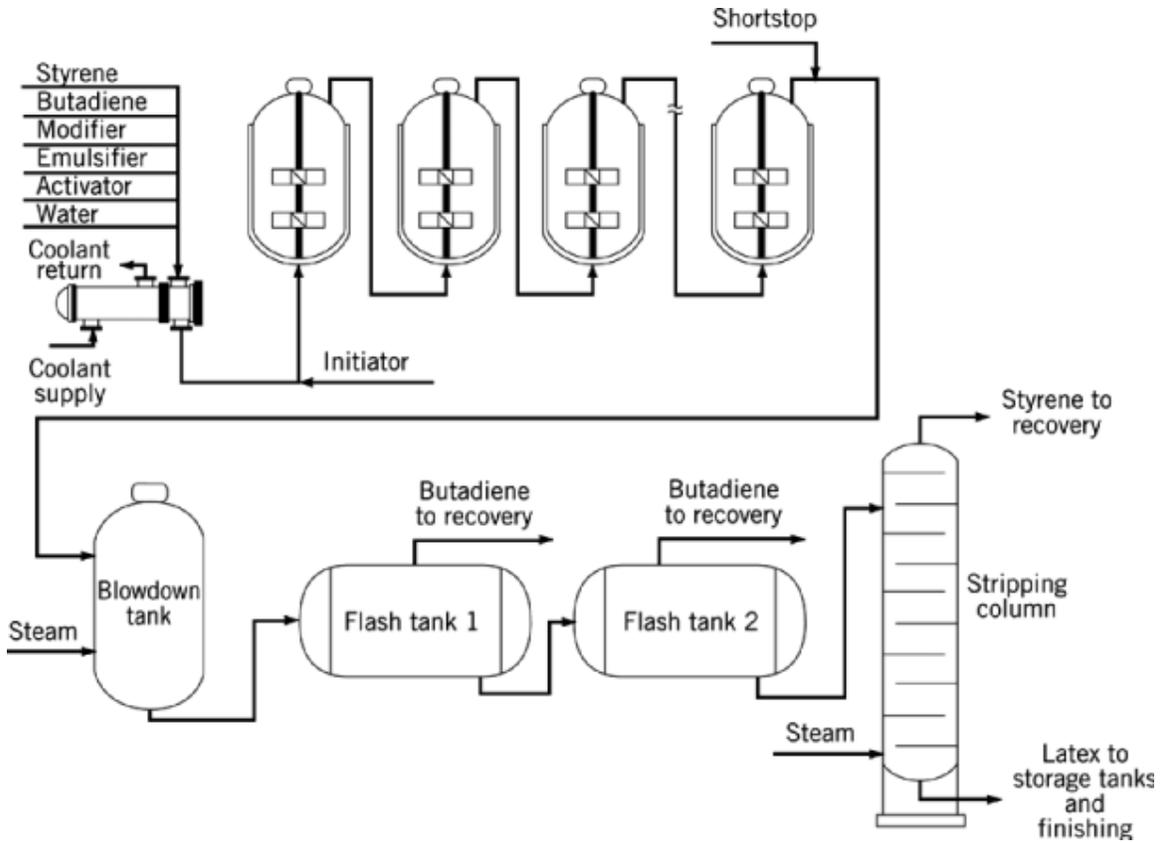


Figure 2-6. Typical continuous emulsion styrene-butadiene rubber polymerization process

Source: Lattime 2000

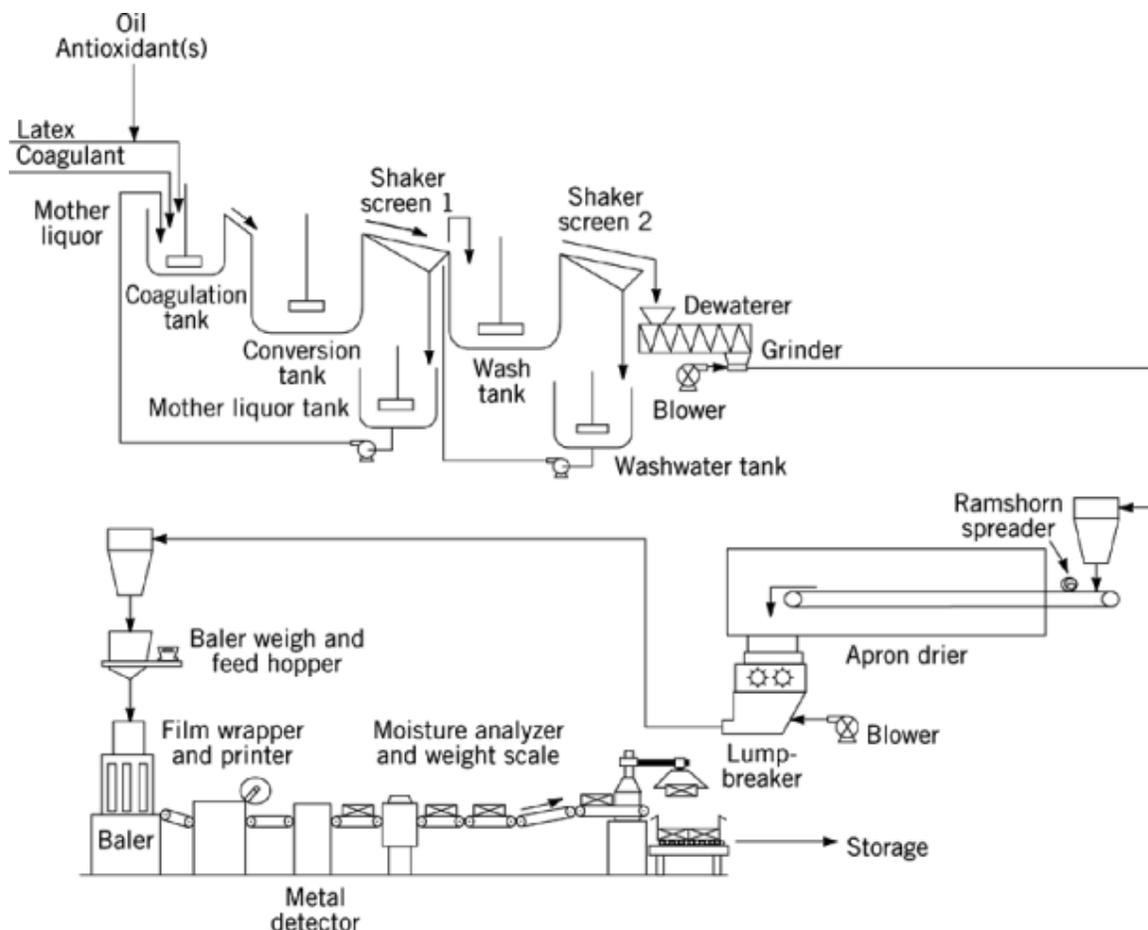


Figure 2-7. Typical emulsion styrene-butadiene rubber finishing process

Source: Lattime 2000

1 2.5.2.2 *Solution process styrene-butadiene rubber production*

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

1 and phosphates, random distribution of the co-monomers is achieved, making it possible
 2 to control polymer composition, monomer distribution sequence, microstructure,
 3 molecular weight, molecular weight distribution, and polymer chain structure. Lattime
 4 (2000) noted that once the ability to control the randomization of solution styrene-
 5 butadiene rubber was better understood and established, it began to displace some of the
 6 market share of emulsion process styrene-butadiene rubber by allowing for more
 7 variation and fine tuning of the styrene-butadiene rubber properties. Figure 2-8 provides a
 8 process flow diagram for solution styrene-butadiene rubber production.

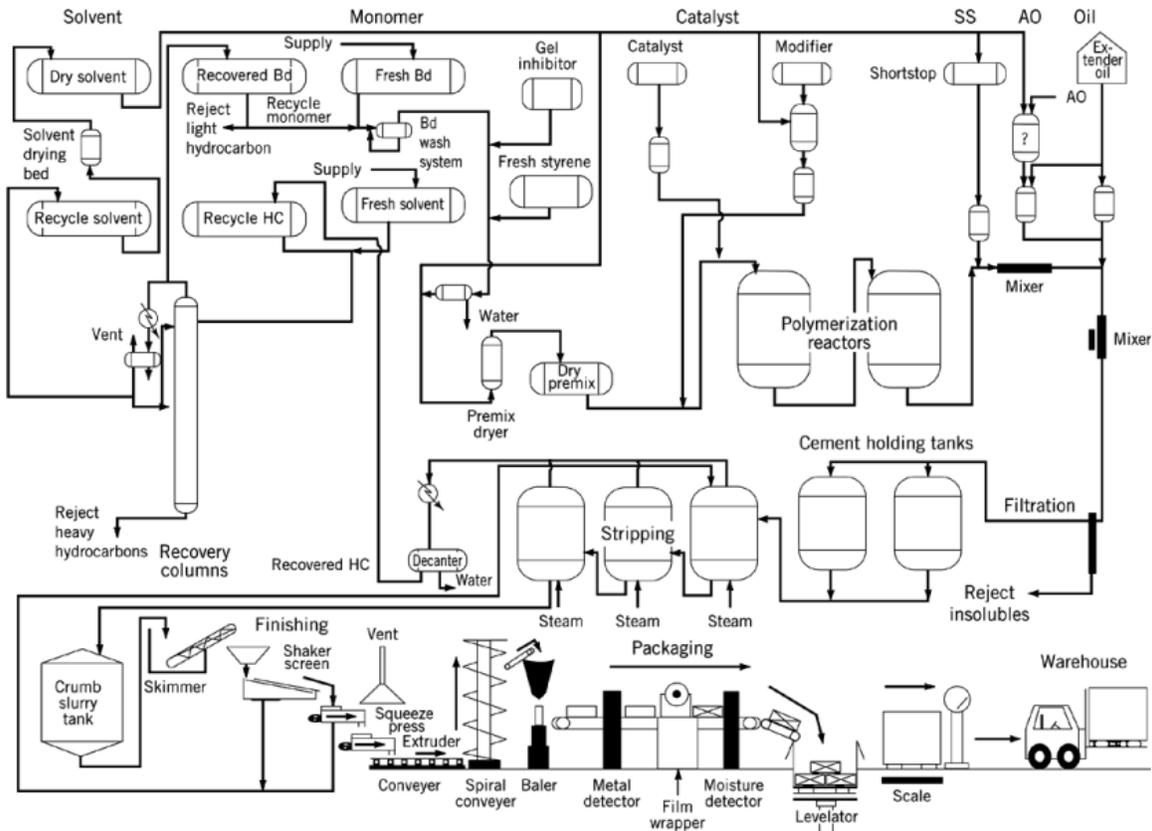


Figure 2-8. Solution styrene-butadiene rubber manufacture by continuous process

Bd=butadiene, HC=hydrocarbon, AO=antioxidant, and SS=shortstop.

Source: Lattime 2000

9 **2.5.3 Styrene-butadiene rubber production exposure levels**

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

1 occur. As can be seen in Table 2-14, mean levels reported for this industry generally are
2 less than 15 ppm. IARC (2002) reported concentrations below 0.15 ppm for vulcanization
3 and extrusion processes involving styrene-butadiene rubber, and exposure to end-users of
4 styrene-butadiene rubber would likely be even lower. Exposure estimates from the series
5 of cancer epidemiology studies of styrene-butadiene rubber production workers in North
6 America (the United States and Canada) (see Section 3.2) are included in Table 2-14. The
7 highest levels of exposure were reported for recovery operators, unskilled maintenance
8 workers, and laboratory technicians (Macaluso *et al.* 2004). Macaluso *et al.* (1996)
9 reported that mean styrene exposure levels declined from approximately 2 ppm in the
10 1940s and 1950s to 0.5 ppm or less in the 1990s; however, workers identified as recovery
11 operators were frequently exposed to levels of 50 ppm or higher during the 1940s and
12 1950s.

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

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

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

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

1 samples were collected from the breathing zone of 28 workers over four months. For
 2 styrene, 336 samples (38%) were below the limit of quantitation (0.007 ppm), 548
 3 samples (62%) were between the limit of quantitation and 20 ppm [which is the Finnish
 4 TLV], and one sample (0.1%) exceeded 20 ppm [actual level not reported]. Mean styrene
 5 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1982 (USA)
Styrene-butadiene rubber plant (NR)	concentrations across five plants	3.53 (0.29–6.66) ^b (N = 3,649)	Matanoski <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 $\mu\text{g}/\text{m}^3$ in source document.

^d Range of mean styrene levels for three factories.

1 2.5.4 The styrene monomer and polymer industry

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

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

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

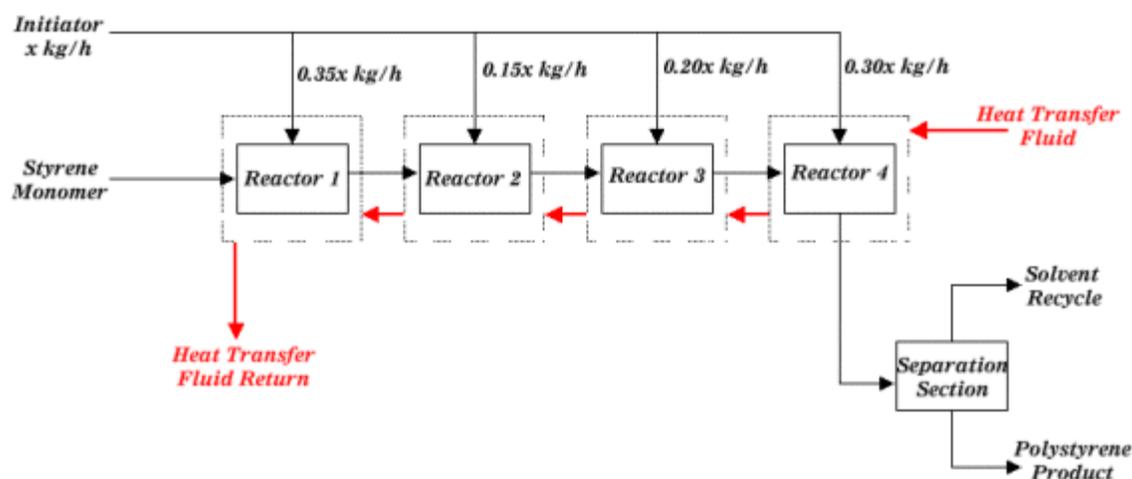


Figure 2-9. Polymerization of polystyrene by the continuous process

Source: Cheresources 2008a.

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

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

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

1 0.6 ppm. It was noted that the highest concentrations occurred during styrene unloading
2 operations (IARC 2002).

3 Some older studies, however, have reported styrene levels in excess of 20 ppm. IARC
4 (2002) presented data from 8-hour personal air samples taken in 1978, 1979, and 1980 in
5 U.S. workplaces where polystyrene and acrylonitrile-butadiene-styrene molding was
6 performed. Styrene levels were 17 to 285 mg/m³ [4.0 to 67 ppm] in 1978, 1.4 to 3.2
7 mg/m³ [0.33 to 0.75 ppm] in 1979, and below the detection limit of 0.01 mg/m³ [0.002
8 ppm] in 1980.

9 Other studies have shown styrene levels that varied mainly with the operations being
10 performed. Based on five separate industrial hygiene surveys conducted between 1962
11 and 1976, Ott *et al.* (1980) reported that TWA exposure levels were below 10 ppm for all
12 jobs in the styrene monomer production industry, including one where excursions were
13 measured as high as 50 ppm during the drumming of styrene. In batch polymerization
14 processes in 1942, styrene levels ranging from 5 to 88 ppm were measured during filling
15 operations; however, subsequent continuous polymerization processes generally resulted
16 in personal exposure measurements of 1 ppm or less. Residual styrene monomer
17 concentrations ranging from less than 1 to 16 ppm have been reported in the vicinity of
18 polystyrene compounding rolls (used for production of sheets of polystyrene) (Ott *et al.*
19 1980).

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

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

1 converted into finished or semi-finished articles. Of the 83 urine samples, 20 were below
 2 the 10 mg/L detection limit, 4 were greater than 500 mg/L and 5 were greater than 1,000
 3 mg/L [concentrations estimated from a graph]. The authors noted that the facilities with
 4 higher styrene air concentrations had a correspondingly higher number of employees with
 5 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 <i>et al.</i> 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 <i>et al.</i> 1980 (NR)
Batch polymerization (1942)	filling operations	NR (5–88)	Ott <i>et al.</i> 1980 (NR)
Polystyrene production (NR)	compounding and rolling	NR (< 1–16)	Ott <i>et al.</i> 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/m³ 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.

1 2.5.5 Other occupational exposures

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

8 Due to the potential VOC production from the irradiation of organic matter and the
9 potential for direct uses and leaks of VOCs during the operation of nuclear power plants,
10 Hsieh *et al.* (2006) investigated the composition and concentrations of a number of VOCs
11 in air-conditioned office space and low-level waste repository sites of three nuclear
12 power plants in Taiwan in 2000. Depending on the type of building being assessed,
13 concentrations were presented only for the 10 or 20 most abundant chemicals. The
14 average styrene level at one low-level waste building was 4.01 ppb by volume (ppbv).
15 Styrene levels were lower in the administrative buildings of the three power plants (0.21,
16 0.65, and 1.07 ppbv). While the authors noted that concentrations of aromatics,
17 chlorofluorocarbons, and chlorinated hydrocarbons were markedly higher in the low-
18 level waste buildings compared with administrative buildings, there was no indication
19 that styrene levels were higher.

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

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

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

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

11 In a study to estimate the level of protection that tollbooths afford workers, Sapkota *et al.*
12 (2005) measured styrene air levels in indoor air and outdoor air of a Baltimore Harbor
13 Tunnel tollbooth in the summer of 2001. For indoor air, the mean styrene concentration
14 was $0.45 \mu\text{g}/\text{m}^3$ [0.11 ppb] with a range of 0.05 to $1.19 \mu\text{g}/\text{m}^3$ [0.01 to 0.28 ppb], and for
15 outdoor levels the mean concentration was $0.61 \mu\text{g}/\text{m}^3$ [0.14 ppb] with a range of 0.05 to
16 $1.68 \mu\text{g}/\text{m}^3$ [0.01 to 0.39 ppb].

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

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

26 In two cooking-ware manufacturing companies where styrene-based resins were used, the
27 8-hour TWA concentrations of styrene ranged from 0.2 to 81 ppm and two short-duration
28 samples were 142 and 186 ppm (IARC 2002). Area samples taken at a college sculpture

1 class where polyester resins were used contained styrene at concentrations from 0.8 to 1.2
2 ppm, and two breathing zone air samples contained 2.8 and 3.0 ppm. In a study of
3 taxidermists who used polyester resins to prepare specimens, air concentrations of styrene
4 ranged from 21 to 300 mg/m³ [4.9 to 70 ppm]. Firefighters can be exposed to styrene
5 during firefighting activities: IARC (2002) reported a level of 1.3 ppm during the
6 knockdown phase of a fire. Styrene air levels exceeding 20 ppm have been reported
7 during the manufacture of polyester paints, lacquers, and putties, and the application of
8 polyester putties during cable splicing operations resulted in exposure levels ranging
9 from 2 to 16 ppm. In a Japanese production plant where buttons were made from
10 polyester resins, 8-hour TWA levels were 7.1 ppm with a maximum air level of 28 ppm.

11 **2.6 Biological indices of exposure**

12 Direct measures of exposure to styrene in humans have included unmetabolized styrene,
13 which has been measured in expired air, blood, and urine (Guillemin and Berode 1988),
14 adipose tissue (Engström 1978), and breast milk (Howard 1989). Although individuals
15 may differ in their ability to metabolize styrene because of differences in metabolizing
16 enzymes resulting from genetic polymorphisms (see Section 5.4.5), metabolites of
17 styrene are widely used as biomarkers of exposure. These metabolites include Phase I
18 intermediates and their conjugates (Phase II intermediates) of styrene glycol and styrene-
19 7,8-oxide in blood; and the urinary biomarkers mandelic acid and phenylglyoxylic acid
20 (IARC 2002), 4-vinylphenol (Manini *et al.* 2003), and phenylhydroxymercapturic acids
21 (PHEMAs) from glutathione conjugation of styrene oxides (Ghittori *et al.* 1997). Finally,
22 adducts of styrene formed through reaction of styrene-7,8-oxide with albumin,
23 hemoglobin, and DNA also have been used as biomarkers of exposure. In contrast with
24 measurements of styrene air concentrations to estimate exposure levels, the use of
25 biological indices will account for exposures from all exposure routes (*i.e.*, inhalation,
26 ingestion, and dermal exposure).

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

1 slow phase for each], while protein adducts have half-lives of one to three months, and
2 DNA adducts have estimated half-lives ranging from 19 hours for the N⁷ DNA adducts to
3 1,320 hours for the O⁶ DNA adduct. The half-lives of styrene, its metabolites, and
4 adducts are discussed further in Section 5.4.

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

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

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

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

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

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

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

21 Other indicators of exposure that have been used include measurements of styrene in
22 urine and styrene-7,8-oxide in blood (HSDB 2008a). Mixed data have been reported on
23 the effectiveness of styrene levels in urine as they relate to exposure levels. Pezzagno *et*
24 *al.* (1985) reported a linear relationship and correlation coefficients for TWA styrene
25 levels in air and styrene in urine of 0.88 (for exposed workers) and 0.93 (for experimental
26 volunteers), while Ong *et al.* (1994) reported a “poor correlation” ($r = 0.24$) between air
27 and urinary styrene levels. Tornero-Velez *et al.* (2001) determined styrene and styrene-
28 7,8-oxide in human blood and reported detection limits of 2.5 $\mu\text{g/L}$ for styrene and 0.05
29 $\mu\text{g/L}$ for styrene-7,8-oxide. The authors reported a linear relationship between levels of
30 styrene in blood and the corresponding air concentrations. Linear regression of logged

1 values yielded the following relationship: $\ln[\text{blood styrene (mg/L)}] = -4.35 + 0.97 \ln[\text{air}$
2 $\text{styrene (ppm)}]$ ($N = 35, r = 0.89$). The authors noted that a styrene exposure level of 50
3 ppm resulted in a level of 0.57 mg/L styrene in blood at the end of a work shift. Levels of
4 styrene-7,8-oxide in the blood were significantly correlated with air levels of both styrene
5 and styrene-7,8-oxide. For styrene-7,8-oxide in blood, linear regression of logged values
6 yielded the following relationship: $\ln[\text{blood SO } (\mu\text{g/L})] = -3.23 + 0.415 \ln[\text{air styrene}$
7 $(\text{ppm})]$ ($N = 27, r = 0.73$). The contribution of dermal styrene exposure was not assessed
8 in these studies.

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

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

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

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

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

20 The measurement of styrene-induced DNA adducts has been reported (Vodicka *et al.*
21 2002a, Vodicka *et al.* 2003, Vodicka *et al.* 2002b) (see Section 5.4), and these adducts
22 have been shown to correlate significantly with measures of styrene exposure, including
23 styrene in workplace air, styrene in exhaled air, styrene in blood, and urinary mandelic
24 acid (see Section 5.4.4, DNA adducts). Vodicka *et al.* (1993) detected 4.7 DNA
25 adducts/ 10^8 nucleotides among 10 hand-lamination workers, and 0.3 adducts/ 10^8
26 nucleotides in 8 controls. Vodicka *et al.* (2002a, 2002b) reported significant linear
27 relationships between styrene exposure and DNA adducts of styrene, *i.e.*, the N²- and O⁶-
28 guanines, with approximately six-fold higher levels of O⁶-guanine DNA adducts in hand-
29 lamination workers as compared with controls. The levels of O⁶-styrene-guanine DNA

1 adducts were significantly correlated with styrene workplace air concentration ($r = 0.588$,
2 $P < 0.001$), duration of employment ($r = 0.479$, $P = 0.002$), and exposure coefficient
3 (workplace air concentration multiplied by years of employment) ($r = 0.659$, $P < 0.001$).
4 Vodicka *et al.* (2003) reported that O⁶-styrene-7,8-oxide–guanine DNA adducts were
5 significantly higher in exposed subjects as compared with controls and were significantly
6 correlated with workplace styrene air concentration ($r = 0.73$, $P < 0.001$) and cumulative
7 exposure ($r = 0.659$, $P = 0.001$). The limit of detection for the DNA adducts was 0.4
8 adducts per 10⁹ nucleotides [0.04 adducts per 10⁸ nucleotides] (Vodicka *et al.* 2003).
9 (Biomarkers of effect (such as DNA repair and toxic endpoints) are discussed in Section
10 5.)

11 Adducts of styrene-7,8-oxide to the *N*-terminal valine in hemoglobin and to cysteine
12 residues in albumin and hemoglobin also have been used as biomarkers of exposure to
13 styrene (Brenner *et al.* 1991, Christakopoulos *et al.* 1993, Fustinoni *et al.* 1998, IARC
14 2002, Liu *et al.* 2001, Yeowell-O'Connell *et al.* 1996, Yuan *et al.* 2007) that can be used
15 to estimate exposure over longer periods of one to three months (Fustinoni *et al.* 1998).
16 Fustinoni *et al.* compared levels of styrene-7,8-oxide adducts of albumin and hemoglobin
17 with the urinary markers of mandelic acid and phenylglyoxylic acid among workers
18 exposed to styrene in the reinforced-plastics industry and in unexposed subjects. They
19 found high levels of albumin and hemoglobin adducts of styrene-7,8-oxide in unexposed
20 controls that were not significantly different from those of the exposed workers. The
21 authors concluded that cigarette smoking is a source of background levels of styrene-7,8-
22 oxide–protein adducts and they suggested that hemoglobin adducts of styrene-7,8-oxide
23 can be detected above background levels only when high-level exposure to styrene exists,
24 which they considered to be 100 mg/m³ [23.5 ppm]. Vodicka *et al.* (2003) reported that
25 *N*-terminal valine adduct levels were significantly higher in exposed subjects as
26 compared with controls and were significantly correlated with workplace styrene air
27 concentration ($r = 0.779$, $P < 0.001$) and cumulative styrene exposure ($r = 0.657$, $P =$
28 0.006). Yeowell-O'Connell *et al.* (1996) found no exposure-related increase in
29 hemoglobin adducts; however, albumin adducts were found to increase with exposure to
30 styrene or styrene-7,8-oxide (the latter being more important). High levels were also
31 found in people without occupational exposure, suggesting to the authors that styrene-

1 7,8-oxide is either produced endogenously or exposure was occurring from other sources
2 (*i.e.*, dietary or other environmental exposures). Significant correlations were found for
3 the styrene-7,8-oxide–albumin adduct 2-phenylethanol versus styrene air levels ($P =$
4 0.017) and styrene-7,8-oxide air levels ($P = 0.01$). These studies did not evaluate the
5 contribution of dermal styrene exposure.

6 **2.7 Regulations and guidelines**

7 *2.7.1 Regulations*

8 **Department of Homeland Security**

9 46 CFR 150 and 151 detail procedures for shipping styrene monomer and for shipping
10 styrene monomer and various styrene co-polymers with incompatible mixtures

11 **Department Of Transportation (DOT)**

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

14 **Environmental Protection Agency (EPA)**

15 Clean Air Act

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

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

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

23 Clean Water Act

24 Styrene has been designated a hazardous substance with a reportable quantity (RQ) of
25 1,000 lb

26 Comprehensive Environmental Response, Compensation, and Liability Act

27 Reportable quantity (RQ) = 1,000 lb

28 Emergency Planning and Community Right-To-Know Act

29 Toxics Release Inventory: Listed substance subject to reporting requirements

1 Safe Drinking Water Act

2 Maximum contaminant level (MCL) = 0.1 mg/L

3 **Food and Drug Administration (FDA)**

4 Maximum permissible level in bottled water = 0.1 mg/L

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

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

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

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

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

20 **Occupational Safety and Health Administration (OSHA)**

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

22 Ceiling concentration = 200 ppm

23 Permissible exposure limit (PEL) = 100 ppm

24 *2.7.2 Guidelines*

25 **American Conference of Governmental Industrial Hygienists (ACGIH)**

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

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

28 Biological exposure indices

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

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

2 **National Institute for Occupational Safety and Health (NIOSH)**

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

4 Short-term exposure limit (STEL) = 100 ppm

5 Recommended exposure limit (REL) = 50 ppm

6 **2.8 Summary**

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

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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).

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

8 In accordance with the IARC evaluation and Cohen *et al.* (2002), this review is organized
9 by the three major industrial settings where workers are exposed to styrene – the
10 reinforced-plastics industry (Section 3.1), the styrene-butadiene rubber industry (Section
11 3.2), and the styrene monomer and polymer industry (Section 3.3) – because exposure
12 conditions differ significantly among these industries. A fourth category includes studies
13 conducted in the general population or other industrial settings (Section 3.4). Section 3.5
14 describes the available case-control and ecological studies. Section 3.6 discusses
15 strengths and limitations of the literature, and Section 3.7 summarizes previous
16 evaluations by IARC (1994a, 2002) and Cohen *et al.* (2002). Section 3.8 summarizes the
17 findings for selected cancer sites. Section 3.9 provides an overall summary for this
18 section.

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

1 **3.1 The reinforced-plastics industry**

2 As noted in Section 2.6.1, the highest occupational exposures to styrene, with respect to
3 the number of employees and exposure levels, occur in the fabrication of objects such as
4 boats, car and truck parts, tanks, tubs, and shower stalls from reinforced plastics (IARC
5 2002). Exposures in this industry have been in the range of several hundred parts per
6 million in the past, but reported levels have declined over the past several decades.
7 Workers in the reinforced-plastics industry may also be exposed to other chemicals,
8 including acetone and other solvents; organic peroxides; cross-linking agents such as
9 methyl methacrylate; chlorinated hydrocarbons such as dichloromethane; hydroquinone;
10 oxidation products such as styrene-7,8-oxide; dusts and fibers (such as glass fibers, silica,
11 asbestos) from filler and reinforcement materials; foaming agents such as isocyanates;
12 and cobalt salts and amines used as accelerators (EPA 1997b, IARC 2002).

13 Cancer mortality or incidence has been studied in the following four populations of
14 reinforced-plastics workers: (1) in Washington state in the United States (Okun *et al.*
15 1985, Ruder *et al.* 2004), (2) in 30 manufacturing plants in unspecified U.S. locations
16 (Wong 1990, 1994), (3) in Denmark (Kolstad *et al.* 1995, Kolstad *et al.* 1993, Kolstad *et*
17 *al.* 1994, Kolstad *et al.* 1996) and (4) in Europe (Denmark [a subset of the workers from
18 the studies by Kolstad *et al.*], Finland, Italy, Norway, the United Kingdom, and Sweden)
19 (Kogevinas *et al.* 1994a, 1993). Results from the U.K. subset of the European population
20 were also reported separately by Coggon *et al.* (1987). The Danish and the European
21 populations were partly overlapping, as 13,682 Danish male workers were included
22 among the 36,610 male workers in the Danish studies reported by Kolstad *et al.* (1995,
23 1994). The two U.S. studies did not overlap. An overview of the individual studies is
24 presented in Table 3-1.

25 **3.1.1 Washington state**

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

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

7 Exposure was assessed using industrial hygiene surveys that classified the jobs and
8 departments according to the level of styrene exposure. According to this assessment,
9 2,060 employees (40%) had ever worked in fibrous glass or lamination departments;
10 these constituted a well-defined high-exposure group. Full-shift average styrene exposure
11 levels within these departments was 42.5 ppm (range 7.3 to 84.7 ppm) at Plant A and
12 71.7 ppm at Plant B (range 14.5 to 183 ppm) in 1978 to 1979 (Crandall 1981). A total of
13 3,141 employees worked with boat assembly, in administration, and in general plant-wide
14 departments with lower styrene exposure levels; these workers were classified as having
15 low exposure and were assigned an exposure level of 5 ppm by the authors; no
16 measurements were reported.

17 For the total cohort, overall cancer mortality was significantly elevated in comparison
18 with Washington state reference rates (SMR = 1.17, 95% CI = 1.02 to 1.33, 233 observed
19 deaths). Statistically significant increases in mortality were also seen for cancer of the
20 esophagus (SMR = 2.30, 95% CI = 1.19 to 4.02, 12 observed deaths), prostate (SMR =
21 1.71, 95% CI = 1.09 to 2.54, 24 observed deaths), and other and unspecified sites (ICD-9
22 codes 194 to 199, SMR = 1.68, 95% CI = 1.01 to 2.62, 19 observed deaths).

23 Among highly exposed workers, a statistically nonsignificant increase in overall cancer
24 mortality was observed (SMR = 1.26, 95% CI = 0.96 to 1.63, 58 observed deaths), as
25 well as statistically nonsignificant increases in mortality due to cancer of the esophagus
26 (SMR = 1.85, 95% CI = 0.22 to 6.67, 2 observed deaths), stomach (SMR = 1.55, 95% CI
27 = 0.19 to 5.61, 2 observed deaths), intestine except rectum (SMR = 1.55, 95% CI = 0.50
28 to 3.63, 5 observed deaths), pancreas (SMR = 1.88, 95% CI = 0.51 to 4.81, 4 observed
29 deaths), lung (SMR = 1.29, 95% CI = 0.76 to 2.04, 18 observed deaths), ovary (SMR =

1 2.32, 95% CI = 0.28 to 8.38, 2 observed deaths), prostate (SMR = 2.06, 95% CI = 0.43 to
2 6.04, 3 observed deaths), kidney (SMR = 3.60, 95% CI = 0.98 to 9.20, 4 observed
3 deaths), bladder (SMR = 3.17, 95% CI = 0.38 to 11.5, 2 observed deaths), and brain
4 (SMR = 1.28, 95% CI = 0.26 to 3.75, 3 observed deaths), and Hodgkin's disease (SMR =
5 1.78, 95% CI = 0.05 to 9.89, 1 observed death). Site-specific mortalities were generally
6 comparable for the low-exposure group except for cancers of the urinary organs, which
7 were higher in the high-exposure group.

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

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

24 The authors stated that the study was limited by lack of information on lifestyle choices,
25 previous or subsequent employment, exposure to other occupational agents, and job
26 information after 1978. Cumulative exposure estimates were not job specific and did not
27 include any exposures between 1978 and when the plant closed (1989 for Plant B and
28 1993 for Plant A). They noted that the lack of job information after 1978 meant that
29 cumulative exposure and duration of exposure are underestimates and would bias results

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

4 3.1.2 *United Kingdom*

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

21 Among all workers at the seven companies with almost complete data, a statistically
22 significant decrease in the SMR for overall cancer mortality was observed (SMR = 0.80
23 (95% CI = 0.69 to 0.93, 167 observed deaths). The SMR did not differ statistically from
24 unity for any specific cancer, but statistically nonsignificant increases (> 10%) in SMRs
25 were observed for larynx (SMR = 1.16, 95% CI = 0.14 to 4.18, 2 observed deaths), lung
26 (SMR = 1.12, 95% CI = 0.89 to 1.39, 83 observed deaths), melanoma (SMR = 1.19, 95%
27 CI = 0.14 to 4.30, 2 observed deaths), non-melanoma skin cancer (SMR = 3.57, 95% CI
28 = 0.43 to 12.90, 2 observed deaths), and cancer of the ovary (SMR = 1.49, 95% CI = 0.41
29 to 3.81, 4 observed deaths). Lung cancer mortality was highest in individuals with
30 moderate and high exposure, and individuals exposed from 1 to 9 years, but the exposure-

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

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

11 3.1.3 *United States*

12 Wong *et al.* reported on cancer mortality among 15,826 workers (75.6% men) at 30 U.S.
13 reinforced-plastics plants between 1948 and 1977 (Wong 1990) and later extended
14 follow-up through 1989 (Wong *et al.* 1994). Employees who had worked for at least 6
15 months in an area with potential exposure to styrene were included in the study.
16 However, not all workers actually worked in activities that entailed direct and significant
17 exposure to styrene (Wong 1990). The duration of employment was less than 1 year for
18 24% of the workers and 5 years or more for 27%. Vital status information was obtained
19 from the participating plants, the Social Security Administration, the National Death
20 Index, the National Center for Health Statistics, and a commercial retail credit bureau.
21 Cause of death was determined from death certificates. Vital status was unknown for 547
22 workers at the end of follow-up, and death certificates were not identified for 42; loss to
23 follow-up was thus 3.7%. Standard SMR analyses were conducted, based on expected
24 values computed from national death rates for whites (no information on race was
25 available for the study population), as well as internal Cox regression analyses. Gender-
26 specific risk estimates were not calculated. Odds ratios (ORs) for respiratory cancer
27 mortality were computed by the Mantel-Haenszel procedure among 40 cases and 102
28 controls nested within the study population. This analysis included information on
29 smoking obtained from 78% of cases and 61% of controls (Wong 1990).

1 A job exposure matrix was constructed for each plant from styrene measurements and
2 process descriptions collected circa 1980. Only 43% of the total study population had
3 direct exposure to styrene, according to information obtained for the 102 controls
4 included in the nested case-control study (Wong 1990). The estimated styrene TWA
5 values by job were 60 ppm (5 to 120 ppm) for spray and hand lay-up; 20 to 45 ppm for
6 sheet molding, gel coating and winding; and 2 to 7 ppm for office, injection molding,
7 field service, finish and assembly, store and ship, and preform production (Wong *et al.*
8 1994). The worker population was then classified into six processing categories based on
9 exposures to styrene and other substances: open-mold processing, mixing and closed-
10 mold processing, finish and assembly, plant office and support, maintenance and
11 preparation, and supervisors and professionals. Cumulative exposure to styrene was
12 estimated, taking account of job changes and duration of employment (Wong *et al.* 1994).

13 For the total study population, the SMR for all cancer was 1.16 (95% CI = 1.05 to 1.27,
14 425 observed deaths) (Wong *et al.* 1994). Statistically significantly increased SMR
15 values were also seen for cancer of the esophagus (SMR = 1.92, 95% CI = 1.05 to 3.22,
16 14 observed deaths), lung (SMR = 1.41, 95% CI = 1.20 to 1.64, 162 observed deaths),
17 cervix (SMR = 2.84, 95% CI = 1.36 to 5.21, 10 observed deaths), and other female
18 genital organs (SMR = 2.02, 95% CI = 1.07 to 3.45, 13 observed deaths). Decreased risks
19 were seen for all lymphatic and hematopoietic malignancies (SMR = 0.82, 95% CI = 0.56
20 to 1.17, 31 observed deaths) and among the subcategories (lymphosarcoma, Hodgkin's
21 disease, leukemia, or cancer of all other lymphopoietic tissue). A statistically
22 nonsignificant increase in mortality from pancreatic cancer was observed (SMR = 1.13,
23 95% CI = 0.68 to 1.77, 19 observed deaths), while mortality from laryngeal cancer was as
24 expected.

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

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

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

21 3.1.4 Denmark

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

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

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

22 Measurements of styrene exposure levels in the industry were available back to the early
23 1960s, based on 2,473 personal air samples collected by the work inspection service.
24 About 90% of the samples were taken during lamination procedures (Jensen *et al.* 1990).
25 The mean period-specific styrene exposure levels were 180 ppm (1964 to 70), 88 ppm
26 (1971 to 75), and 43 ppm (1976 to 88), and an estimated 43% of the study population
27 were laminators (Kolstad *et al.* 1994).

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

1 95% CI = 0.93 to 1.05, 1,134 observed cases) (Kolstad *et al.* 1995), and a statistically
2 nonsignificant increase in the incidence of all lymphohematopoietic malignancies was
3 observed (SIR = 1.20, 95% CI = 0.98 to 1.44, 112 observed cases) (Kolstad *et al.* 1994).
4 Analyses of specific cancers revealed no statistically significantly increased SIRs
5 (Kolstad *et al.* 1995, 1994). Incidences were nonsignificantly increased for cancer of the
6 pancreas (SIR = 1.20, 95% CI = 0.86 to 1.63, 41 observed cases), nasal cavities (SIR =
7 1.84, 95% CI = 0.74 to 3.80, 7 observed cases), lung (SIR = 1.12, 95% CI = 0.98 to 1.26,
8 248 observed cases), pleura (SIR = 1.78, 95% CI = 0.85 to 3.28, 10 observed cases),
9 external male genital organs (SIR = 1.60, 95% CI = 0.64 to 3.30, 7 observed cases), and
10 bladder (SIR = 1.16, 95% CI = 0.96 to 1.39, 117 observed cases) (Kolstad *et al.* 1995),
11 NHL (SIR = 1.33, 95% CI = 0.96 to 1.80, 42 observed cases), and leukemia (SIR = 1.22,
12 95% CI = 0.88 to 1.65, 42 observed cases) (Kolstad *et al.* 1994).

13 The risks of cancers with elevated SIRs were further evaluated by an internal analysis
14 using workers not exposed to styrene as controls. The category with the highest potential
15 styrene exposure was workers at companies with 50% to 100% laminators. Among these
16 workers, there was a statistically significant excess of pancreatic cancer (IRR = 2.2, 95%
17 CI = 1.1 to 4.5, 17 observed cases) (Kolstad *et al.* 1995). The risk was higher in long-
18 term workers (≥ 1 year) than in short-term workers and among workers with earlier first
19 years of employment (1970 or before) than later; however, latency had no influence on
20 IRR values. The risk of lung cancer was not increased (IRR = 1.0, 95% CI = 0.7 to 1.3,
21 72 cases) among workers with higher exposure potential, and lower risk was seen in
22 long-term workers. Analyses by first year of employment, length of employment, and
23 latency revealed no consistent findings for cancer of the nasal cavities, pleura, external
24 male genital organs, or urinary bladder, NHL or the other lymphohematopoietic
25 malignancies except for leukemia (Kolstad *et al.* 1994, 1995).

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

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

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

13 3.1.5 Denmark, Finland, Norway, Italy, Sweden, and the United Kingdom.

14 Kogevinas *et al.* (1994a, 1993) reported on cancer mortality among 40,688 employees at
15 660 companies in Denmark (15,867), Finland (2,085), Norway (2,035), Italy (7,256),
16 Sweden (3,667), and the United Kingdom (9,778). Cancer mortality among 7,971 of the
17 U.K. workers was previously reported by Coggon *et al.* (1987) but with a shorter follow-
18 up. The international study included the male (13,682) workers that were also included in
19 the cancer incidence studies of the Danish population (Kolstad *et al.* 1995, Kolstad *et al.*
20 1994) and the the female (2,185) workers that were not included in those studies from
21 287 Danish plants where reinforced plastics were the main products produced. The
22 follow-up periods started between 1945 (United Kingdom) and 1970 (Denmark) and
23 ended between 1987 (Sweden) and 1991 (Norway). The duration of employment was less
24 than 2 years for 60% of the workers and 10 years or more for 9%. Loss to follow-up was
25 1.4%, and the average follow-up was 13 years. In SMR analyses, cancer mortality was
26 compared with expected mortality computed from national reference rates. In internal
27 analyses, Poisson regression models were used to compare exposure-specific cancer rates
28 (rate ratios) and conduct trend analyses.

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

1 with bystander exposure (5,406), workers not exposed to styrene (4,044), and workers
2 with unknown job titles (1,201). The 15,867 Danish workers were categorized as workers
3 with unspecified tasks, because no job titles were available. An exposure matrix was
4 constructed from 16,500 personal styrene measurements obtained between 1955 and
5 1990 and from 18,500 measurements of styrene metabolites in urine conducted in the
6 1980s. Styrene exposure levels averaged across country, period, and job were linked with
7 the individual workers, and cumulative styrene exposure was estimated from additional
8 information on duration of exposure. All Danish workers and other workers classified as
9 having unspecified tasks were assigned a styrene exposure level that was the average
10 value for the calendar period and branch of the industry (boats vs. other). Styrene
11 exposure levels recorded among laminators declined from about 200 ppm before 1965 to
12 below 80 ppm in the 1980s.

13 Among the total study population, overall cancer mortality was statistically significantly
14 lower than in the European reference population (SMR = 0.87, 95% CI = 0.81 to 0.94,
15 686 observed deaths). This was also the case for cancer of the buccal cavity and pharynx
16 (SMR = 0.33, 95% CI = 0.11 to 0.77, 5 observed deaths), rectum (SMR = 0.62, 95% CI =
17 0.38 to 0.95, 21 observed deaths), breast (SMR = 0.52, 95% CI = 0.28 to 0.89, 13
18 observed deaths), and brain (SMR = 0.62, 95% CI = 0.37 to 0.98, 18 observed deaths).
19 No site-specific SMR values were statistically significantly above unity, but excesses
20 were seen for small intestine (SMR = 1.50, 95% CI = 0.31 to 4.38, 3 observed deaths),
21 larynx (SMR = 1.11, 95% CI = 0.53 to 2.05, 10 observed deaths), ovary (SMR = 1.40,
22 95% CI = 0.70 to 2.51, 11 observed deaths) and myeloid leukemia (SMR = 1.10, 95% CI
23 = 0.63 to 1.79, 16 observed deaths) (Kogevinas *et al.* 1994a).

24 The worker category with the best-documented high-level styrene exposure was
25 laminators. Laminators showed no statistically significant elevated mortality from cancer
26 at any site, but statistically nonsignificant increases in mortality were observed for cancer
27 of the esophagus (SMR = 1.82, 95% CI = 0.87 to 3.34, 10 observed deaths), small
28 intestine (SMR = 2.27, 95% CI = 0.06 to 12.66, 1 death), pancreas (SMR = 1.48, 95% CI
29 = 0.76 to 2.58, 12 observed deaths), larynx (SMR = 1.55, 95% CI = 0.32 to 4.52, 3
30 deaths), ovary (SMR = 2.61, 95% CI = 0.71 to 6.69, 4 observed deaths), and thyroid

1 (SMR = 2.27, 95% CI = 0.06 to 12.66, 1 observed death), NHL (SMR = 1.40, 95% CI =
2 0.56 to 2.88, 7 observed deaths), and Hodgkin's disease (SMR = 1.33, 95% CI = 0.27 to
3 3.88, 3 observed deaths) (Kogevinas *et al.* 1994a).

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

8 Lower SMRs were observed for long-term workers (≥ 2 years) for all
9 lymphohematopoietic malignancies, Hodgkin's disease, multiple myeloma, and
10 leukemia, but not for NHL (< 2 years: SMR = 0.60, 95% CI = 0.19 to 1.40, 5 observed
11 deaths; ≥ 2 years: 1.05, 95% CI = 0.42 to 2.17, 7 observed deaths). More than 20 years
12 after first exposure, a statistically nonsignificant increase in mortality for NHL in long-
13 term workers was observed (SMR = 2.21, 95% CI = 0.45 to 6.45, 3 observed deaths).

14 Among workers exposed for at least 2 years, mortality was non-significantly elevated for
15 all lymphohematopoietic malignancies (SMR = 1.73, 95% CI = 0.70 to 3.57, 7 observed
16 deaths) and leukemia (SMR = 1.94, 95% CI = 0.40 to 5.66, 3 deaths) in workers with at
17 least 20 years latency, but not in those with latency of 10 to 19 years (Kogevinas *et al.*
18 1994a).

19 In internal analyses, the relative risk increased with increasing latency for all
20 lymphohematopoietic malignancies ($P = 0.012$), leukemia ($P = 0.094$), and malignant
21 lymphoma (NHL and Hodgkin's disease, $P = 0.072$). Similarly, the rate ratio increased
22 with increasing average styrene exposure for all lymphohematopoietic cancers (P for
23 linear trend = 0.019) and for malignant lymphoma ($P = 0.052$), though not for leukemia
24 ($P = 0.47$). A trend for increased risk of pancreatic cancer with increasing cumulative
25 styrene exposure was of borderline significance ($P = 0.07$), but no such trend was seen
26 for all cancer, cancer of esophagus, lung, or kidney, all lymphohematopoietic
27 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 <i>et al.</i> 1985 Ruder <i>et al.</i> 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>Industry hygiene surveys</i> Workers employed in fibrous glass or lamination departments (40% of population) classified as highly exposed. <i>Exposure Levels</i> <u>High-exposure workers</u> Average TWA (ppm) 1978–79 (Crandall 1981) Plant A: 42.5 Plant B: 71.7 <u>Low-exposure workers</u> 5 ppm (assigned not measured)	<i>Total cohort</i> <u>Cancers with excess mortality (significant)</u> 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 <i>High-exposure workers</i> <u>Cancers with non-significant excess mortality</u> 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 <i>SMR for lung cancer no longer elevated in high exposed workers exposed for > 1 yr</i> Statistically significant elevated SMRs were also observed for cancers of the esophagus, prostate and unspecified sites in the low exposed workers <u>Urinary tract cancer</u> <i>Trend towards increasing SMR with increasing</i>	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
				<i>duration of exposure, and level (tercile) of exposure</i>	
Coggon <i>et al.</i> 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	<i>Total cohort (7 of the 8 companies)^d</i> 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 <i>Laminators</i> <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 <i>et al.</i> (1994a)
Wong 1990 U.S.	historical cohort 1948–77 nested case-control study	<i>Historical cohort</i> 15,908 workers employed ≥ 6 mo in 30 reinforced-plastics companies in work	<i>Cohort study</i> Work histories were obtained from employment records. An industrial hygiene survey, which	<i>Cohort study: Respiratory cancer</i> 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	<p>areas with exposure to styrene 1948–77; 499 deaths were observed</p> <p>SMRs based on national rates</p> <p><i>Nested case-control study</i></p> <p><i>Cases:</i> 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</p> <p><i>Controls:</i> 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</p> <p>ORs calculated by Mantel-Haenszel, and logistic regression methods</p>	<p>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.</p> <p><i>Case-control study</i></p> <p>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.</p>	<p>TWA)</p> <p><i>Subgroups with excess respiratory cancers</i></p> <p>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.</p> <p>Higher SMRs were observed in workers in hot process than cold process</p> <p>(See later update [Wong <i>et al.</i> 1994] for findings on cancer at other sites)</p> <p><i>Case-control study of respiratory cancer</i></p> <p><u>Mantel-Haenszel OR, cases/controls, P</u> styrene (direct) exposure 0.63, 15/44, 0.29</p> <p><u>Logistic regression</u></p> <p>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.</p>	<p>cohort were not used in the case-control analysis</p> <p>Potential exposure to styrene is higher for cold process than hot process</p>
Wong <i>et al.</i>	historical cohort	Same cohort as Wong 1990, except 15,826	Company-specific JEMs constructed from	<i>Total cohort (1,628 deaths)</i>	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	<p><u>Cancers with significant excess mortality</u></p> <table border="0"> <tr> <td>all cancer</td> <td>1.16 (1.05–1.27), 425</td> </tr> <tr> <td>esophagus</td> <td>1.92 (1.05–3.22), 14</td> </tr> <tr> <td>cervix</td> <td>2.84 (1.36–5.21), 10</td> </tr> <tr> <td>other female</td> <td></td> </tr> <tr> <td> genital organs</td> <td>2.02 (1.07–3.45), 13</td> </tr> <tr> <td>lung</td> <td>1.41 (1.20–1.64), 162</td> </tr> </table> <p>No exposure-response relationship (cumulative exposure or duration) observed for any cancer</p> <p><u>Lung cancer</u></p> <p><i>Latency (years since first employment)</i></p> <table border="0"> <tr> <td>< 10</td> <td>1.07, 23, <i>P</i> > 0.05</td> </tr> <tr> <td>10–19</td> <td>1.46, 70, <i>P</i> < 0.01</td> </tr> <tr> <td>≥ 20</td> <td>1.51, 69, <i>P</i> < 0.01</td> </tr> </table> <p><u>Internal analysis (stepwise regression and multivariate); entire cohort</u></p> <p>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</p> <p><i>High-exposure workers (open mold processing category) with > 2 years of exposure</i></p> <p>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</p>	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	< 10	1.07, 23, <i>P</i> > 0.05	10–19	1.46, 70, <i>P</i> < 0.01	≥ 20	1.51, 69, <i>P</i> < 0.01	<p>population was exposed to high styrene levels</p> <p>Cox regression models of cumulative styrene exposure may have been over-controlled by the inclusion of duration of employment</p> <p>SMRs for high-exposure workers based on small numbers of observed and expected deaths</p>
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																						
< 10	1.07, 23, <i>P</i> > 0.05																						
10–19	1.46, 70, <i>P</i> < 0.01																						
≥ 20	1.51, 69, <i>P</i> < 0.01																						
Kolstad <i>et al.</i>	historical	36,610 workers at 386	No data on individual	<i>Total cohort (Kolstad et al. 1994 LH, 1995, solid</i>	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 <i>et al.</i> 1993, Kolstad <i>et al.</i> 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 <i>et al.</i> 1990)	<p><i>tumors</i>) all solid cancers 0.99 (0.93–1.05), 1,134</p> <p><u>Cancers with non-significantly elevated SIRs</u></p> <p>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</p> <p><u>Leukemia, by employment variables (Kolstad <i>et al.</i> 1994)</u></p> <p><i>Probable exposure</i> high 1.38 (0.75–2.32), 14 low 1.15 (0.77–1.67), 28</p> <p><i>Year of first employment</i> 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</p> <p><i>Years since first employment</i> < 10 0.71 (0.34–1.31), 10 ≥ 10 1.57 (1.07–2.22), 32</p> <p><i>Years of employment (≥ 10 years latency)</i> < 1 2.34 (1.43–3.61), 20 ≥ 1 1.01 (0.52–1.77), 12</p> <p><u>Internal analysis (probable high exposure)</u></p>	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 <i>et al.</i> (Kogevinas <i>et al.</i> 1994a, Kogevinas <i>et al.</i> 1993)

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

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 <i>et al.</i> 1994a, Kogevinas <i>et al.</i> 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	<i>Total cohort</i> Cancers with non-significant excess mortality (> 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 <i>Laminators</i> 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 <i>et al.</i> 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 <i>Poisson regression exposure models for cancer</i> Test for trend in RRs: <i>P</i> -value <i>Average exposure</i> LH 0.019 leukemia 0.47 malignant lymphoma 0.052 <i>Time since first exposure</i> LH 0.012 leukemia 0.094 malignant lymphoma 0.072 <i>Cumulative exposure</i> no increase in RR with increasing exposure for any LH cancer type <i>Pancreas</i> RR increased with increasing cumulative exposure (<i>P</i> = 0.068) <i>Esophagus, or kidney</i> RR increased (non-significantly) with cumulative exposure (slightly for esophagus) <i>Lung</i> 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.

^aThe 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.

^bUnless otherwise stated.

^cNot including rectum.

^dRecords and follow-up from one company were incomplete, so the analysis for that company was reported separately.

1 **3.2 The styrene-butadiene rubber industry**

2 Generally lower styrene exposure levels are seen in the styrene-butadiene rubber industry
3 than the glass fiber–reinforced plastics industry, although significant exposures to
4 workers can still occur (see Section 2.6). Mean levels reported for this industry are
5 generally less than 15 ppm for synthesis of styrene-butadiene latex, and concentrations
6 below 0.15 ppm have been reported for vulcanization and extrusion processes involving
7 styrene-butadiene rubber. Exposure to end-users (such as rubber tire manufacturers)
8 would likely be even lower. Workers in the styrene-butadiene industry can be exposed to
9 1,3-butadiene and DMDTC, in addition to styrene (Delzell *et al.* 2001, Macaluso *et al.*
10 2004). This section does not include studies on end-users except for McMichael *et al.*
11 (1976a) because it provides specific estimates for workers in a plant producing styrene
12 butadiene (primarily) and other rubbers.

13 McMichael *et al.* (1976a) studied cancer mortality at a rubber tire manufacturing plant in
14 the United States. Meinhardt *et al.* (1982, 1978) and Lemen *et al.* (1990) initially studied
15 2,756 workers at two styrene-butadiene rubber plants (forming one complex) in Texas.
16 Matanoski and coworkers (Matanoski *et al.* 1997, Matanoski *et al.* 1993, Matanoski *et al.*
17 1990, Matanoski and Schwartz 1987, Santos-Burgoa *et al.* 1992) studied workers (from
18 12,110 to 13,686, depending on study)² in eight other styrene-butadiene rubber plants
19 (seven U.S. plants and one Canadian plant). Later, Delzell and colleagues (Delzell *et al.*
20 1996, 2001, 2006, Macaluso *et al.* 2004, 1996; Sathiakumar *et al.* 1998, 2005, and Graff
21 *et al.* 2005) studied 13,130 to 16,610 styrene-butadiene rubber industry workers from the
22 same plants studied by Meinhardt *et al.* and seven of the plants studied by Matanoski,
23 Santos-Burgoa, and coworkers (Delzell *et al.* 2001, Delzell *et al.* 1996). Delzell *et al.*,
24 Macaluso *et al.* (1996, 2004), Sathiakumar *et al.* (1998, 2005), and Graff *et al.* (2005) did
25 not have access to information from the previous studies by Meinhardt *et al.*, Matanoski
26 *et al.*, and Santos-Burgoa *et al.* that allowed identification of individual subjects and a
27 formal evaluation of the overlap between the populations. The study populations were
28 established by different procedures and exclusion criteria, which may partly explain the

² Number of workers varied among the publications.

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

3 **3.2.1 United States: McMichael et al.**

4 McMichael *et al.* (1976a) studied 6,678 male workers at a rubber tire manufacturing
5 plant. A small fraction of the total cancer cases (2% with 5 or more years of employment
6 and 3% with 2 or more years of employment) was observed among workers engaged in
7 the production of styrene-butadiene and other synthetic rubbers; however, the proportion
8 of the total cohort exposed to synthetic rubbers was not given. The total population was
9 followed from 1964 to 1972, deaths were identified, and work histories were compared
10 between cases and an age-stratified random sample of all workers (22%) in internal
11 analyses. Work histories were extracted from personnel records and grouped into 16
12 major work areas. One of these areas was work in the synthetic plant where styrene-
13 butadiene rubber was produced. For workers with at least 5 years employment in the
14 synthetic plant, significantly increased risk ratios were observed for stomach cancer (RR
15 = 2.2 , 99.9% CI = 1.4 to 4.3, number of deaths not stated), lymphohematopoietic cancer
16 (RR = 6.2, 99.9% CI = 4.1 to 12.5), and lymphatic leukemia (RR = 3.9, 99.9% CI = 2.6
17 to 8.0). No other significant associations between work in synthetic rubber production
18 and other selected cancers were observed. [Note: exposure ratios and/or risk ratios were
19 reported by the authors only for certain selected cancer sites (see Table 3-9). It appears
20 that if an exposure ratio greater than 1 was observed, an age-standardized risk ratio (in
21 comparison with all workers in the cohort) was then calculated and reported.]

22 **3.2.2 United States: Meinhardt et al.**

23 Meinhardt *et al.* studied the mortality of 2,756 styrene-butadiene rubber workers at two
24 plants in Texas, prompted by the deaths of 2 workers from leukemia (Meinhardt *et al.*
25 1982, 1978). White male workers with at least six months of non-management or non-
26 administrative employment were included in the study. The population was followed
27 from 1943 through 1976, and 3% were lost to follow-up. SMR analyses compared
28 observed deaths with expected values computed from the national rates. The average
29 length of employment was about 10 years. A total of 53,929 person-years were
30 accumulated, and the average follow-up was 19 years. Average TWA styrene exposure

1 levels based on samples from all areas of the production facilities were 0.94 ppm (0.03 to
2 6.46) and 1.99 ppm (0.05 to 12.3) for the two plants.

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

14 Lemen *et al.* (1990) reported on a further follow-up of this cohort through December 31,
15 1982 at one plant (A) and December 31, 1981 for a second plant (B), yielding a total of
16 43,341 person-years at risk of death and 390 observed deaths, 77 from cancers, at plant A
17 and 26,314 person-years and 148 observed deaths, 29 from cancers, at plant B. In the
18 subcohort exposed to the batch production process used between 1943 and 1945, a total
19 of 19,582 person-years and 291 observed deaths, 61 from cancers, were available for
20 analysis. No SMRs for cancers were reported in this follow-up report. However, the
21 authors noted that mortality for cancers of the trachea, bronchus, and lung had increased
22 (from 16 to 34 deaths) and that the only other increases in SMRs were observed for
23 lymphosarcoma and reticuloma (3 deaths in the first analysis and 5 deaths in the follow-
24 up; these 2 additional deaths occurred in the subcohort exposed to the batch process, as
25 did the one additional lymphohematopoietic death in this follow-up.

26 3.2.3 *United States and Canada: Matanoski, Santos-Burgoa, and coworkers*

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

1 Matanoski *et al.* 1993, Matanoski *et al.* 1990, Matanoski and Schwartz 1987, Santos-
2 Burgoa *et al.* 1992). The number of workers included in the different publications
3 differed slightly, from 12,110 (Matanoski *et al.* 1997, Matanoski *et al.* 1993, Matanoski
4 *et al.* 1990), to 13,686 (Santos-Burgoa *et al.* 1992), or 13,920 (Matanoski and Schwartz
5 1987). The population was initially followed to 1979 (Matanoski and Schwartz 1987) and
6 was updated through 1982 (Matanoski *et al.* 1990). Loss to follow-up was 3%, and
7 251,431 person-years were accumulated; the average follow-up was about 21 years.
8 Mortality was compared with national rates, and SMR values were calculated (Matanoski
9 *et al.* 1990, Matanoski and Schwartz 1987). In internal analyses of lymphohematopoietic
10 malignancies, odds ratios were estimated by Mantel-Haenszel methods and by
11 conditional and unconditional regression analysis (Matanoski *et al.* 1997, Santos-Burgoa
12 *et al.* 1992). Initial internal analyses relied on 59 cases and 193 controls individually
13 matched by plant (and other variables) (Santos-Burgoa *et al.* 1992). Subsequent analyses
14 included 58 cases and replaced the original controls with 1,242 controls sampled without
15 individual matching (Matanoski *et al.* 1997). This was done to avoid over-matching,
16 because measurements indicated that styrene exposure levels differed between plants.

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

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

1 Among all workers at the eight plants, overall cancer mortality was less than expected
2 (SMR = 0.85, 95% CI = 0.78 to 0.93, 518 observed deaths). Statistically nonsignificant
3 increases in mortality were observed for Hodgkin's disease (SMR = 1.20, 95% CI = 0.52
4 to 2.37, 8 observed deaths) and for other lymphatic malignancies (SMR = 1.11, 95% CI =
5 0.64 to 1.77, 15 observed deaths). No increased mortality was reported for the other
6 lymphohematopoietic cancers, including leukemia (Matanoski *et al.* 1990). Mortality
7 from all lymphopoietic [lymphohematopoietic] malignancies was not related to duration
8 of employment. SMR values were also presented separately for white and black
9 production workers, maintenance workers, utility workers, and other workers, but it was
10 not clear whether styrene exposure differed among these categories.

11 Among workers categorized as having a cumulative styrene exposure score above
12 average, the internal matched analyses presented by Santos-Burgoa *et al.* (1992) showed
13 statistically nonsignificant increases in the odds ratios for leukemia (OR = 3.13, 95% CI
14 = 0.84 to 11.2, 26 cases, 84 controls), lymphosarcoma (OR = 1.33, 95% CI = 0.11 to
15 16.6, 6 cases, 23 controls), and other lymphatic malignancies (mainly myeloma) (OR =
16 1.35, 95% CI = 0.25 to 7.40, 18 cases, 56 controls), but a non-significant decrease in the
17 OR for Hodgkin's disease (OR = 0.40, 95% CI = 0.05 to 3.25, 8 cases, 29 controls).
18 Comparable ORs were seen in unmatched analyses of the same dataset. In matched
19 models that controlled for exposure to butadiene, no increased risk was apparent for
20 leukemia (OR = 1.06, 95% CI = 0.23 to 4.95), or other lymphatic malignancies (OR =
21 0.94, 95% CI = 0.16 to 5.53); the OR for all lymphohematopoietic malignancies was 1.29
22 (95% CI = 0.53 to 3.15). There was no indication of positive interaction between
23 exposure to styrene and butadiene for all lymphohematopoietic malignancies; however,
24 no results were obtained for leukemia, because the model did not converge.

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

1 myeloma (OR = 3.04, 95% CI = 1.33 to 6.96, 10 deaths), lymphomas (OR = 2.67, 95%
2 CI = 1.22 to 5.84, 12 deaths), and all lymphohematopoietic malignancies (OR = 2.20,
3 95% CI = 1.46 to 3.33, 58 deaths), but not for leukemia (no estimate was provided for
4 leukemia). [Note that styrene alone remained in the final model for each of these
5 cancers.] Also, final models for leukemia and Hodgkin's disease (which were not
6 associated with styrene exposure) included only exposure to butadiene. With respect to
7 cumulative exposure, using the same initial variables, the mortality for leukemia
8 increased statistically significantly by increasing cumulative styrene exposure ($P = 0.006$)
9 in a final model in which both butadiene exposure and duration of employment remained.
10 Styrene alone was also significantly associated with myeloma ($P = 0.023$) and with all
11 lymphohematopoietic cancers ($P = 0.000$ [P -value as reported in the paper]) in a final
12 model in which styrene and duration of employment remained. [Final models for
13 Hodgkin's disease included butadiene exposure and duration. Note that the ICD codes for
14 lymphomas (200 & 202) are the same as non-Hodgkin's lymphoma.]

15 3.2.4 United States and Canada: Delzell, Sathiakumar, Macaluso, Graff

16 Delzell *et al.* (1996) established a cohort of all 17,964 male workers employed for at least
17 one year between 1943 and 1990 at eight U.S. styrene-butadiene rubber plants (two of
18 these plants were organized as one company complex) and one Canadian styrene-
19 butadiene rubber plant. (The start date for some of the plants varied between 1950 and
20 1965.) The population included workers at seven of the eight plants previously studied by
21 Matanoski *et al.* (1993, 1990) and Santos-Burgoa *et al.* (1992) and at the two-plant
22 complex studied by Meinhardt *et al.* (1982). The companies employed an estimated total
23 of 25,500 workers. [Note that the Delzell cohort expanded the cohort to include more
24 recent employees with start dates up to 1990, whereas the earlier cohorts followed
25 workers employed from 1943 to 1976. Adding workers with lower exposures, shorter
26 latency, and duration worked might reduce apparent risk.]

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

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

6 3.2.4.1 Study population

7 The first study was conducted among workers employed for at least one year between
8 1943 and 1990 and followed up until 1991 (Delzell *et al.* 1996, Sathiakumar *et al.* 1998);
9 at that time, 25% had died (3,976), 70% were presumed alive, and 5% were lost to
10 follow-up. Mortality analysis was conducted on 15,649 subjects and excluded 2,315
11 Canadian workers who had not worked in styrene-butadiene rubber or other related
12 operations or who had worked in unspecified areas of one of the plants. Delzell *et al.*
13 2006 reported that the “2,312” Canadian workers had worked in butyl rubber production
14 or styrene production or were unspecified. Follow-up was later extended to 1998 for the
15 cohort of 17,924 workers, and included 6,327 observed deaths while vital status was
16 unknown for 570 (3%) (Delzell *et al.* 2006, Sathiakumar *et al.* 2005). The authors stated
17 that 40 workers from the original cohort were excluded because they did not meet study
18 criteria (employment length or gender), or were duplicates, [but they did not discuss the
19 2,315 non-styrene-butadiene rubber workers excluded from the first study]. Analyses
20 based on work areas were limited to workers in styrene-butadiene rubber operations (N =
21 15,612).

22 Different subsets of the cohort were used in the series of studies of quantitative estimates
23 of styrene exposure and lymphohematopoietic cancer mortality (primarily leukemia)
24 (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.* 2005, Macaluso *et al.* 1996). The
25 analyses reported by Macaluso *et al.* (1996) and Delzell *et al.* (2001) were based on the
26 1991 follow-up, and analyses reported by Graff *et al.* and Delzell *et al.* 2006 were based
27 on the 1998 follow-up. The study by Macaluso *et al.* (1996) (which evaluated cumulative
28 exposure to styrene, butadiene and mortality from leukemia) excluded 1,354 workers (of
29 the 17,964 member cohort) at two plants for whom quantitative estimates of exposure
30 could not be established, and the analyses were based on 16,610 workers. Delzell *et al.*

1 (2001) further excluded twelve workers with duplicate records and 3,468 workers who
2 died before reaching 40 years of age or 10 years latency, because no leukemia deaths
3 occurred in these groups; this left 13,130 workers. This study also used a revised
4 exposure assessment (see below) and evaluated leukemia mortality and quantitative
5 exposure to styrene, butadiene, and DMDTC. The study reported by Graff *et al.* (2005)
6 and Delzell *et al.* (2006) stated that their analysis was on 16,579 workers for whom
7 quantitative estimates of exposure could be established (which excluded 25 workers
8 (from Macaluso *et al.* 2006) who were determined to have duplicate records or did not
9 meet study criteria such as employment length or gender).

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

16 3.2.4.2 *Exposure assessment and job classification*

17 Personnel records were reviewed, and 308 work-area groups or job groups with similar
18 tasks and exposure potential were identified. The groups were further combined into five
19 main process groups and seven process subgroups: (1) rubber/butadiene production
20 (37%): polymerization, coagulation, finishing; (2) maintenance (24%): shop, field;
21 (3) labor (15%): production, maintenance; (4) laboratories (9%); and (5) other operations
22 (15%) (Sathiakumar *et al.* 1998). Macaluso *et al.* (1996) constructed a plant-specific, job-
23 exposure matrix from industrial hygiene monitoring surveys, archival material, walk-
24 through surveys, meetings with plant officials, and interviews with workers. Eight-hour
25 TWA exposure levels for styrene, butadiene, and benzene were estimated for each year
26 between 1943 and 1992 for each of the work areas or job groups by air dispersion models
27 (Macaluso *et al.* 1996). Cumulative exposure was computed, taking into account the
28 extents and durations of different tasks. As of the end of the 1991 follow-up, 83% of the
29 cohort were considered to have been exposed to styrene, with a median cumulative

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

3 Delzell *et al.* (2001) later characterized this exposure estimation process as controversial,
4 and they revised the exposure estimates and added estimates of exposure to DMDTC, as
5 further described by Macaluso *et al.* (2004). The authors did not substantiate this critique,
6 but others have cited them as characterizing the original estimates as uncertain and not
7 completely validated (Sielken and Valdez-Flores 2001). [The revised exposure estimates
8 used by Macaluso *et al.* (2004) represented an improvement over the original estimates
9 because of detailed industrial hygiene and chemical engineering reviews of the processes,
10 job activities and work area, and historical changes. A senior industrial hygienist, who
11 had extensive experience within the industry and with the methodology for estimating
12 historical exposures, guided the work, which included identification of new tasks,
13 additional information on the operations, modification of some of the assumptions needed
14 to estimate exposure, and verification that all of the assumptions were reasonable.
15 Information on use of personal protective equipment was obtained through interviews
16 with long-term employees.] According to the original exposure assessment, estimated
17 TWA styrene exposure levels for active workers declined from 1.8 ppm in the 1940s to
18 0.1 ppm in the early 1990s (Macaluso *et al.* 2004), partly because of decreasing exposure
19 levels and partly because of decreasing styrene exposure prevalence. The revised styrene
20 TWA exposure estimates were about twice as high as the original estimates reported in
21 Macaluso *et al.* 1996 and declined from about 2 ppm during 1940 to 1970 to about
22 0.5 ppm in the late 1980s. The revised assessment estimated a median cumulative
23 exposure of 17 to 18 ppm-years (Delzell *et al.* 2001, Macaluso *et al.* 2004) for the 85% of
24 the workers who were exposed to styrene. The cumulative styrene exposure estimates
25 were highly correlated with those for butadiene and DMDTC (Spearman rank
26 correlations of 0.78 and 0.60, respectively). Note that 79% of workers were estimated to
27 have been exposed to butadiene and 62% to DMDTC (Delzell *et al.* 2001). Exposure to
28 DMDTC occurs primarily through dermal absorption, and cumulative estimated exposure
29 was calculated as mg-years DMDTC/cm of skin.

1 3.2.4.3 SMR analyses

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

5 SMR analyses based on follow-up until 1991 showed that among all styrene-butadiene
6 rubber workers, overall cancer mortality was less than expected (SMR = 0.93, 95% CI =
7 0.87 to 0.99, 950 observed deaths) (Sathiakumar *et al.* 1998). Significant deficits in
8 mortality were also seen for cancer of the buccal cavity and pharynx (SMR = 0.50, 95%
9 CI = 0.28 to 0.82, 15 observed deaths) and esophagus (SMR = 0.59, 95% CI = 0.35 to
10 0.93, 18 observed deaths). A statistically nonsignificant excess of mortality from
11 leukemia was seen among all workers (SMR = 1.31, 95% CI = 0.97 to 1.74, 48 observed
12 deaths), and significant excesses were seen among all workers ever employed hourly
13 (SMR = 1.43, CI not reported, $P < 0.05$, 45 deaths) and among ever-hourly workers with
14 employment duration of at least 10 years and latency of at least 20 years (SMR = 2.24,
15 95% CI = 1.49 to 3.23, $P < 0.05$, 28 deaths) (Sathiakumar *et al.* 1998). Increased
16 leukemia mortality was also seen among workers in polymerization (SMR = 2.51, 95%
17 CI = 1.40 to 4.14, 15 deaths), coagulation (SMR = 2.48, 95% CI = 1.00 to 5.11, 7
18 observed deaths), the maintenance subgroup of labor (SMR = 2.65, 95% CI = 1.41 to
19 4.53, 13 observed deaths), and laboratories (SMR = 4.31, 95% CI = 2.07 to 7.93, 10
20 observed deaths) (Delzell *et al.* 1996).

21 Repeated SMR analyses based on the extended follow-up until 1998 did not change this
22 mortality pattern considerably (Sathiakumar *et al.* 2005). All cancer mortality (SMR =
23 0.92, 95% CI = 0.88 to 0.97, 1,608 observed deaths), and buccal cavity-pharynx cancer
24 mortality (SMR = 0.47, 95% CI = 0.29 to 0.71, 22 observed deaths), still showed
25 significant deficits, while this no longer was the case for esophageal cancer (SMR = 0.94,
26 95% CI = 0.68 to 1.26, 44 observed deaths). With respect to lymphohematopoietic
27 cancers, non-significant excesses in mortality were observed for leukemia (SMR = 1.16,
28 95% CI = 0.91 to 1.47, 71 observed deaths) and Hodgkin's disease. All
29 lymphohematopoietic malignancies, NHL, and multiple myeloma showed observed
30 numbers of death close to the expected (Sathiakumar *et al.* 2005). Sub-analyses indicated

1 statistically nonsignificant increases in leukemia in workers ever employed hourly (SMR
2 = 1.23, 95% CI = 0.94 to 1.57, 63 observed deaths), and significant excesses among those
3 employed for at least 10 years and 20 to 29 years since hire (SMR = 2.58, 95% CI = 1.56
4 to 4.03, 19 observed deaths). No excess of leukemia was seen for 30 years or more after
5 first employment (SMR = 1.02, 95% CI = 0.62 to 1.58, 20 observed deaths).

6 Several analyses of cell-type specific leukemias were conducted by Sathiakumar *et al.*
7 (2005), Graff *et al.* (2005), and Delzell *et al.* (2006) including acute lymphocytic
8 leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia
9 (AML), chronic myelogenous leukemia (CML) and other leukemias and NHL+CLL. A
10 statistically significant increase in SMR for CML was observed among the ever-hourly
11 employed workers (SMR = 2.00, 95% CI = 1.00 to 3.58, 11 observed deaths)
12 (Sathiakumar *et al.* 2005). A statistically nonsignificant increase in mortality from CLL
13 was observed (SMR = 1.71, 95% CI = 0.96 to 2.81, 15 observed deaths), while there was
14 no increase in the numbers of AML (SMR = 0.97, 95% CI = 0.48 to 1.73, 11 observed
15 deaths) or ALL (SMR = 0.51, 95% CI = 0.01 to 2.82, 1 observed death). Statistically
16 significant increases were observed for AML among workers with < 20 years since hire
17 and < 10 years employment (SMR = 4.78, 95% CI = 1.30 to 12.24, 4 deaths) and for
18 CML (SMR = 6.55, 95% CI = 2.4 to 14.26, 6 deaths). Significant (or borderline)
19 increases were also found for NHL+CLL among every-hourly workers (SMR = 1.30,
20 95% CI = 0.99 to 1.67, 60 observed deaths), workers with 20 to 29 years since hire and
21 10+ years employment (SMR = 1.90, 95% CI = 1.01 to 3.25, 13 observed deaths), and
22 with 30+ years since hire and 10+ years employment (SMR = 1.49, 95% CI = 1.02 to
23 2.10, 32 observed deaths).

24 With respect to work area and job type, statistically significant excesses of all leukemias
25 were observed for workers employed in polymerization (SMR = 2.04, 95% CI = 1.21 to
26 3.22, 18 observed deaths), coagulation (SMR = 2.31, 95% CI = 1.11 to 4.25, 10 observed
27 deaths), maintenance labor (SMR = 2.03, 95% CI = 1.14 to 3.35, 15 observed deaths) and
28 laboratories (SMR = 3.26, 95% CI = 1.78 to 5.46, 14 observed deaths) (which appear to
29 be due primarily to increases in CLL in the same departments; the SMRs for CLL were
30 4.97 (95% CI = 2.15 to 9.80, 8 observed deaths) in polymerization; 6.07 (95% CI = 1.97

1 to 14.17, 5 observed deaths), for coagulation; 3.09 (95% CI = 0.84 to 7.92, 4 observed
2 deaths) in maintenance labor; and 5.59 (95% CI = 1.52 to 14.31, 4 observed deaths) in
3 laboratories. SMR also were increased for AML (SMR = 2.95, 95% CI = 0.96 to 6.88, 5
4 observed deaths) in maintenance labor, CML in laboratories (SMR = 5.22, 95% CI = 1.08
5 to 15.26, 3 observed deaths), and CLL among finishers (SMR = 3.44, 95% CI = 1.38 to
6 7.09, 7 observed deaths). (The authors noted that while workers were assigned to one
7 department for these analyses, there was a considerable likelihood of overlap between
8 various departments (Sathiakumar *et al.* 2005, Delzell *et al.* 2006). Significant increases
9 were also observed for NHL+CLL among workers in polymerization (SMR = 2.18, 95%
10 CI = 1.31 to 3.41, 19 observed deaths) and finishing (SMR = 1.91, 95% CI = 1.21 to
11 2.86, 23 observed deaths).

12 Graff *et al.* (2005) and Delzell *et al.* (2006) also reported SMRs, adjusted for age, race,
13 and calendar year, for all leukemias, CLL, AML, CML, and other leukemias, NHL, and
14 multiple myeloma (MM), by cumulative level of styrene exposure. Statistically
15 significant increases in SMRs were observed in the two highest categories of styrene
16 exposure for all leukemias: SMR = 1.87 (95% CI = 1.02 to 3.13, 14 observed deaths) for
17 31.8 to < 61.1 ppm-years, and SMR = 1.91 (95% CI = 1.09 to 3.10, 16 observed deaths)
18 for 61.1+ ppm-years. Statistically significant increases also were seen for the highest
19 category of exposure only for NHL (SMR = 1.97, 95% CI = 1.08 to 3.31, 14 observed
20 deaths), CLL (SMR = 3.10, 95% CI = 1.01 to 7.24, 5 observed deaths), and for the
21 highest category for NHL+CLL (SMR = 2.29, 95% CI = 1.36 to 3.62, 18 observed
22 deaths).

23 3.2.4.4 Internal analyses of leukemia and other lymphohematopoietic cancers

24 Among the 15,649 workers studied by Delzell *et al.* (1996), 48 deaths with leukemia as
25 the underlying diagnosis had been identified as of the end of follow-up in 1991. [This
26 analysis excluded the 2,315 non-styrene-butadiene rubber workers.] Macaluso *et al.*
27 (1996) included 58 leukemia deaths (7 with leukemia as a contributory diagnosis and 51
28 with leukemia as an underlying diagnosis on the death certificate) identified among
29 16,610 workers followed up to 1991. In a later analysis, Delzell *et al.* (2001) added 1
30 decedent with myelodysplasia as the underlying cause of death and a medical record that

1 indicated leukemia. In the follow-up of 17,924 men to 1998, Sathiakumar *et al.* (2005)
2 identified a total of 162 lymphohematopoietic cancers: 53 NHL, 12 Hodgkin's disease,
3 71 leukemia and 26 multiple myeloma based on the underlying cause of death. In the
4 analysis of 16,579 workers followed to 1998 for whom quantitative exposure estimates
5 were available, Graff *et al.* (2005) identified 81 deaths from leukemia, 58 from NHL, 27
6 from multiple myeloma, and 13 from Hodgkin's disease, with these diagnoses as the
7 underlying or contributing cause of death and confirmed by medical records, if available.
8 (Note: Delzell *et al.* 2006 stated that the death certificate diagnoses and ICD codes (e.g.,
9 71 leukemias) were used for the external analysis to avoid information bias). Relative
10 risks were computed by Poisson regression models (Delzell *et al.* 2001, Delzell *et al.*
11 2006, Graff *et al.* 2005, Macaluso *et al.* 1996) or the Mantel-Haenszel method (Macaluso
12 *et al.* 1996).

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

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

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

4 Delzell *et al.* (2001) presented RRs for leukemia mortality by cumulative exposure to
5 styrene based on the revised exposure assessment. As mentioned previously, the analysis
6 excluded workers who died before reaching 40 years of age or 10 years latency because
7 no deaths from leukemia occurred before this age and length of employment. Controlling
8 for age and time since hire, Delzell *et al.* also found an increasing trend of leukemia
9 deaths with cumulative styrene exposure. The findings were as follows: 0 ppm-years
10 (reference group), RR = 1.0; > 0 to < 20.6 ppm-years, RR = 1.2 (95% CI = 0.5 to 3.3);
11 20.6 to < 60.4 ppm-years, RR = 2.3 (95% CI = 0.9 to 6.2); ≥ 60.4 ppm-years, RR = 3.2
12 (95% CI = 1.2 to 8.8). This trend was reduced when butadiene exposure was introduced
13 to the model; the findings were as follows: 0 ppm-years (reference group), RR = 1.0; > 0
14 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
15 (95% CI = 0.4 to 6.4); ≥ 60.4 ppm-years, RR = 1.8 (95% CI = 0.4 to 7.3). If analyses
16 furthermore included DMDTC exposure, no increasing trend was seen; the findings were
17 as follows: 0 ppm-years, RR = 1.0; > 0 to < 20.6 ppm-years, RR = 0.6 (95% CI = 0.1 to
18 2.5); 20.6 to < 60.4 ppm-years, RR = 0.8 (95% CI = 0.2 to 3.7); ≥ 60.4 ppm-years, RR =
19 0.8 (95% CI = 0.2 to 3.8).

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

1 (< 38.7 ppm-years) according to the revised exposure estimates, but this category
2 included only 4 workers with styrene exposure above the reference category (Delzell *et*
3 *al.* 2001). On the other hand, among workers classified with the highest cumulative
4 butadiene exposure (≥ 287.3 ppm-years), the risk of leukemia increased with cumulative
5 styrene exposure; the findings were as follows: 10.4 to 40.5 ppm-years, RR = 2.6 (95%
6 CI = 0.7 to 9.2, 3 observed deaths); ≥ 40.6 ppm-years, RR = 4.1 (95% CI = 2.0 to 8.4, 18
7 observed deaths). No deaths from leukemia occurred in the reference category (< 10.4
8 ppm-years).

9 Graff *et al.* (2005) repeated these analyses based on the revised exposure assessment by
10 (Delzell *et al.* 2001) and the 81 leukemia deaths observed up to the end of follow-up in
11 1998 (Graff *et al.* 2005). In addition, Graff *et al.* analyzed these leukemias by subtype:
12 CLL (25 deaths); AML (including myelogenous and monocytic leukemias) (26 deaths);
13 CML (16 deaths), and other leukemias (14 deaths). Graff *et al.* also analyzed findings for
14 other lymphohematopoietic cancers, including NHL (58 deaths), multiple myeloma (27
15 deaths), and Hodgkin's disease (13 deaths). Detailed descriptions of the methods and full
16 models included in these analyses were reported in Delzell *et al.* 2006. Findings for the
17 single-chemical, two-chemical and three-chemical models and leukemia, NHL and
18 NHL+CLL are present in Tables 3-2 and 3-3. [Note that no trend analyses were
19 performed for most of these models.]

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

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

3 These analyses were also repeated using total styrene peaks (> 50 ppm) and total
 4 butadiene peaks (> 100 ppm). The single-chemical (styrene) model showed an increasing
 5 trend with exposure compared with zero exposure (See Table 3-4). Intermediate and
 6 three-chemical models also showed an increasing trend with styrene exposure, but in
 7 each model the level of risk was somewhat attenuated (Graff *et al.* 2005, Delzell *et al.*
 8 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.

^aPeak 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).

^bModels were adjusted for age and years since hire.

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

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

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

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

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

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

26

Table 3-3. Cumulative exposure to styrene, butadiene 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.

^a8 deaths had double diagnosis of CLL and NHL.

^bModels 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 <i>et al.</i> 1976a U.S.	Internal case-control comparison of a cohort of rubber workers 1964–72	<i>Cohort</i> 6,678 male workers at a rubber tire plant that produced styrene-butadiene rubber (SBR) and other rubbers <i>Case-control comparison</i> 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) <i>Workers producing SBR and other synthetic rubbers with at least 5 years of exposure (significant findings)</i> LH lymphatic leukemia Stomach cancer 6.2 (4.1–12.5) 3.9 (2.6–8.0) 2.2 (1.4 – 4.3) <i>Number of deaths for all 16 work areas (deaths specifically for the synthetic plant not reported)</i> 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 <i>et al.</i> 1982, Meinhardt <i>et al.</i> 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	<i>Total cohort</i> all cancer 0.72 (0.54–0.93), 56 <u>Cancers with non-significant excess mortality</u> LH NHL leukemia 1.32 (0.66–2.37), 11 1.65 (0.45–4.27), 4 1.73 (0.63–3.73), 6	Study initiated in response to leukemia deaths of 2 workers Study population included in studies by Delzell <i>et al.</i>

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths) ^a	Comments
Lemen <i>et al.</i> 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 <i>et al.</i> 1997, Matanoski <i>et al.</i> 1993, Matanoski <i>et al.</i> 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	<p><i>Total cohort</i></p> <p>all cancer 0.85 (0.78–0.93), 518</p> <p><u>Cancers with non-significant excess mortality</u></p> <p>Hodgkin’s disease 1.20 (0.52–2.37), 8 other lymphatic 1.11 (0.64–1.77), 15</p> <p><i>Production workers</i></p> <p><u>Cancers with excess mortality (non-significant and significant)</u></p> <p>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</p>	Most of the study population included in studies by Delzell <i>et al.</i>
Santos-Burgoa <i>et al.</i> 1992 U.S. and Canada	nested case-control study of LH malignancies	<p><i>Cohort:</i> Matanoski and Schwartz 1987, Matanoski <i>et al.</i> 1990</p> <p><i>Cases:</i> 59 workers who died of LH malignancies</p> <p><i>Controls:</i> 193 workers from cohort who were alive or had died of non-cancer causes; matched to cases by</p>	<p>JEM created by experts based on job titles and descriptions</p> <p>Workers classified according to ranks for relative exposure to styrene and 1,3-butadiene</p> <p>Cumulative exposure calculated based on exposure</p>	<p>OR (95% CI)</p> <p><i>Cumulative styrene exposure > average</i></p> <p><u>Matched analysis</u></p> <p>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)</p>	<p>Styrene exposure ranks correlated poorly with measurements of styrene</p> <p>Styrene exposure and butadiene exposure were positively</p>

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) <u>Unmatched analysis</u> 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 <i>et al.</i> 1997	nested case-control study of LH malignancies	<p><i>Cohort:</i> Matanoski and Schwartz 1987, Matanoski <i>et al.</i> 1990</p> <p><i>Cases:</i> 58 workers who died of LH cancer (starting with the same 59 cases as Santos-Burgoa <i>et al.</i>)</p> <p><i>Controls:</i> 1,242 workers from cohort selected to represent distribution across plants and with similar age distribution to cases</p> <p>ORs calculated with unconditional regression models (controls, N = 1,242); multivariate models included birth year, hire date, and employment duration</p>	<p>Mean styrene exposure for all plants = 3.53 ppm (SD = 14.32), based on 3,649 measurements (1978–83) (Matanoski <i>et al.</i> 1993)</p> <p>Plant means = 0.29–6.66 ppm</p> <p>Styrene levels averaged across jobs and plants</p> <p>Average and cumulative exposure calculated from plant info., job title, and exposure duration</p>	<p>OR (95% CI), no. of observed deaths</p> <p>Logistic regression analysis controlling for butadiene exposure risk</p> <p><i>Increase of 1 ppm in TWA styrene exposure (significant associations)</i></p> <p>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</p> <p><i>Cumulative exposure to styrene</i></p> <p><u>Increasing risks with increasing exposure</u></p> <p>LH <i>P</i> = 0.000^c leukemia <i>P</i> = 0.006 myeloma <i>P</i> = 0.023</p>	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.			
<p>Delzell <i>et al.</i> 1996, Sathiakumar <i>et al.</i> 1998 Sathiakumar <i>et al.</i> 2005, extended follow-up Delzell <i>et al.</i> 2006 U.S. and Canada</p>	<p>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</p>	<p>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</p>	<p>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 <i>Extended follow-up based on identical exposure characterization as the initial</i></p>	<p><i>Follow-up 1943–91:</i> <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 <i>Follow-up 1944–98:</i> <u>Cancers with significantly decreased</u></p>	<p>Includes workers from Meinhardt <i>et al.</i> 1982 (2^c plants) and Matanoski <i>et al.</i> 1990 (7 of 8 plants) Mortality analysis of this cohort (or subpopulations) also published by Macaluso <i>et al.</i> 1996, Delzell <i>et al.</i> 2001, and Graff <i>et al.</i> 2005.</p>

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths) ^a	Comments
			<i>studies.</i>	<p><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</p> <p><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</p> <p><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</p> <p>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</p> <p><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)</p>	

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 <i>et al.</i> 1996	historical cohort 1943–92 418,846 person years	Cohort established by Delzell <i>et al.</i> 1996 <i>Mortality analysis</i> 16,610 workers at 6 of 8 plants (with specific work histories), including workers involved in SBR-unrelated activities <i>External analysis</i> SMRs based on national rates 51 deaths from leukemia <i>Internal analysis</i> 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	<i>Plant-specific JEMs:</i> 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	<i>Leukemia</i> SMR (external analysis) and RR (adjusted for race, age, and cumulative exposure to butadiene) for cumulative exposure to styrene <table border="1" data-bbox="1203 672 1556 862"> <thead> <tr> <th>ppm-years</th> <th>SMR</th> <th>RR</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0.89</td> <td>1.0</td> </tr> <tr> <td>< 5</td> <td>0.63</td> <td>0.9</td> </tr> <tr> <td>5–9</td> <td>1.61</td> <td>5.4</td> </tr> <tr> <td>10–39</td> <td>1.36</td> <td>3.4</td> </tr> <tr> <td>≥ 40</td> <td>2.35</td> <td>2.7</td> </tr> </tbody> </table> <i>Internal analysis</i> test for trend in RR, $P = 0.14$	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	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
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																					

Study	Study design & follow-up	Study population and methods	Exposure	Effects (SMR (95% CI), no. of observed deaths) ^a	Comments
Delzell <i>et al.</i> 2001	historical cohort 1943–91	Cohort established by Delzell <i>et al.</i> 1996 Mortality analysis 13,130 workers from 6 of 8 plants (similar to Macaluso <i>et al.</i> 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 Macaluso <i>et al.</i> 1996	Exposure estimates by Macaluso <i>et al.</i> 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 <i>Leukemia</i> <i>Styrene exposure (ppm-years)</i> <u>Styrene only</u> 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 <u>Styrene + butadiene</u> 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) <u>Styrene + butadiene + DMDTC</u> 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 <i>et al.</i> 1996
Graff <i>et al.</i> 2005; Delzell <i>et al.</i> 2006	historical cohort 1943–98 500,174 person-years	Cohort established by Delzell <i>et al.</i> 1996 <i>Mortality analysis:</i> 16,579 ^g workers at 6 of 8 plants (similar to Macaluso <i>et al.</i> 1996) RR calculated by Poisson	Revised exposure estimates by Macaluso <i>et al.</i> 2004 were used Individuals assigned to four quartiles of cumulative exposure to styrene (ppm-years):	<i>Internal RR analyses:</i> 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																					
		<p>regression models for exposure to single agents and exposure to multiple agents; models included age, latency, period of employment (year range), and race</p> <p>SMRs based on U.S. or Ontario rates for expected numbers of LH cancer deaths</p> <p>81 deaths from leukemia, 58 from NHL, 27 from multiple myeloma, and 13 from Hodgkin's disease</p>	<p>0–< 8.3 8.3–< 31.8 31.8–< 61.1 ≥ 61.1</p> <p>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):</p> <p><i>low</i>: no exposure + 1st quartile <i>medium</i>: 2nd + 3rd quartiles <i>high</i>: 4th quartile</p>	<p>hire) – See data in Table 3-2 and 3-3 for all leukemia, NHL and NHL+CLL.</p> <p>CLL alone by tertiles 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)</p> <p>No statistically significant increases in RR were reported for CLL, AML, CML, other leukemias, or multiple myeloma (single- and 3-chemical models)</p> <p>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.</p> <p>Analysis of cross-classified categories of butadiene and styrene; reference group = low styrene, low butadiene</p> <p><u>RR for leukemia and styrene exposure</u></p> <p>Low-butadiene exposure:</p> <table border="0"> <tr> <td>medium</td> <td>1.6 (0.7–3.9)</td> <td>7</td> </tr> <tr> <td>high</td> <td></td> <td>no deaths</td> </tr> </table> <p>Medium-butadiene exposure</p> <table border="0"> <tr> <td>low</td> <td>1.2 (0.4–3.1),</td> <td>5</td> </tr> <tr> <td>medium</td> <td>1.6 (0.9–2.9),</td> <td>25</td> </tr> <tr> <td>high</td> <td>1.6 (0.6–4.0),</td> <td>6</td> </tr> </table> <p>High-butadiene exposure</p> <table border="0"> <tr> <td>low</td> <td></td> <td>no deaths</td> </tr> <tr> <td>medium</td> <td>3.5 (1.3–9.3),</td> <td>5</td> </tr> </table>	medium	1.6 (0.7–3.9)	7	high		no deaths	low	1.2 (0.4–3.1),	5	medium	1.6 (0.9–2.9),	25	high	1.6 (0.6–4.0),	6	low		no deaths	medium	3.5 (1.3–9.3),	5	<p>Correlation of styrene exposure with butadiene = 0.7 and with DMDTC = 0.63</p>
medium	1.6 (0.7–3.9)	7																								
high		no deaths																								
low	1.2 (0.4–3.1),	5																								
medium	1.6 (0.9–2.9),	25																								
high	1.6 (0.6–4.0),	6																								
low		no deaths																								
medium	3.5 (1.3–9.3),	5																								

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 <u>RR for styrene (ppm-year exposure) adjusted for BD</u> 8.3 – < 61.1 1.5 (0.8-2.8) 61.1+ 1.4 (0.6-3.0) Test for trend: <i>P</i> = 0.65 <i>External analysis</i> 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.

^aUnless otherwise stated.

^b Number of workers varied among the publications.

^c*P*-value as reported in paper.

^dDelzell *et al.* (Delzell *et al.* 1996) reported mortality from LH cancer, whereas Sathiakumar *et al.* (1998) reported mortality from all cancer.

^eDelzell *et al.* (1996) referred to these two plants as one facility.

^fReported 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.

^gCombines the results for 16 men who had worked in different plants and had separate records and eliminates 8 men who had worked < 1 year.

1 **3.3 The styrene monomer and polymer industry**

2 Styrene exposure levels in the styrene monomer and polymer production industries are
3 generally much lower than levels in the reinforced-plastics industry, and are similar in
4 magnitude to levels seen in the styrene-butadiene rubber production industry (see Section
5 2.6). Although levels exceeding 20 ppm have been reported in polymerization,
6 manufacturing, and purification areas for this industry, the styrene levels in maintenance,
7 laboratory, and packaging operations were generally less than 5 ppm. Workers in the
8 styrene monomer industry can also be exposed to benzene, toluene, ethylbenzene, and
9 other alkylbenzene compounds. In addition to benzene, toluene, and ethylbenzene,
10 workers in polystyrene production can be exposed to various solvents such as 1,2-
11 dichloroethane, carbon tetrachloride, ethyl chloride, methylene dichloride, and
12 chlorobenzene. Workers could also be exposed to boron trifluoride which is the preferred
13 initiator for the polymerization reaction (see Section 2.2). Cancer mortality for workers in
14 the styrene monomer and polymer industry has been studied in workers in Germany by
15 Frentzel-Beyme *et al.* (1978), in the United States by Ott *et al.* (1980) (with follow-up by
16 Bond *et al.* (1992) and by Nicholson *et al.* (1978), and in England by Hodgson and Jones
17 (1985). Table 3-5 provides an overview of the studies conducted in the styrene monomer
18 and polymer industry.

19 **3.3.1 Germany**

20 Frentzel-Beyme *et al.* (1978) studied 1,960 workers (sex not reported) engaged in the
21 manufacture of styrene and styrene polymer for more than 1 month during the period
22 1931 to 1976. The population was identified from plant records and followed from 1956
23 through 1976. Percentage follow-up was much lower for non-German employees (29%),
24 many of whom returned to their home countries, compared with German employees
25 (93%). Observed numbers of cancer deaths were compared with the expected numbers
26 based on regional mortality rates. A total of 20,138 person-years were accumulated, and
27 the average follow-up was 10.3 years. Styrene exposure levels were generally below 1
28 ppm according to measurements conducted in 1975 and 1976 (Thiess and Friedheim
29 1978). Levels up to 6.84 ppm (styrene production) and 46.92 ppm (polystyrene
30 production) occasionally were recorded. No subclassification of workers was done that
31 allowed any assessment of cancer mortality by indicators of styrene exposure level. Only

1 12 deaths due to malignant tumors were observed compared with 18.5 expected. A
2 statistically nonsignificant increase in mortality from pancreatic cancer was observed (2
3 observed deaths vs. 0.7 expected; $P = 0.16$), and mortality from lung cancer was
4 decreased (3 observed deaths vs. 5.4 expected; P value not reported). Non-significant
5 increases in mortality from rectal, peritoneal, and splenic cancer were also observed, but
6 these increases were based on only one observed case for each site.

7 3.3.2 United States- multi-plant

8 Ott *et al.* (1980) studied 2,904 male workers employed for at least 1 year in the
9 production or research units of a company that produced styrene monomer, styrene-
10 butadiene latex, and styrene-based products at several U.S. locations. The workers were
11 identified from annual census lists for 1937 to 1970 and followed from 1940 through
12 1976. Bond *et al.* (1992) extended follow-up to 1986 and only 0.4% were lost to follow-
13 up. Vital status and cause of death were assessed using the company mortality
14 surveillance system. Mortality was compared with expected numbers calculated from
15 national rates and other worker populations within the company. A total of 90,000
16 person-years were accumulated, and average follow-up was 31 years. Industrial
17 hygienists assigned all manufacturing jobs (categorized into 57 groups with common
18 exposures) an exposure intensity with respect to five chemical exposures: (1) styrene and
19 ethylbenzene (1 to 4 ppm, or ≥ 5 ppm), (2) benzene, alkylbenzene compounds (≥ 1 ppm),
20 (3) styrene, ethylbenzene, and acrylonitrile in equal concentrations (1 to 4 ppm, or ≥ 5
21 ppm), (4) extrusion fumes, and (5) colorants (indirect and direct exposure).

22 For the total study population, overall cancer mortality was significantly decreased
23 (SMR = 0.81, 95% CI = 0.69 to 0.95, 162 observed deaths) (Bond *et al.* 1992). Increased
24 (but statistically nonsignificant) SMRs were seen for all lymphatic and hematopoietic
25 malignancies (SMR = 1.44, 95% CI = 0.95 to 2.08, 28 observed deaths), Hodgkin's
26 disease (SMR = 2.22, 95% CI = 0.71 to 5.18, 5 observed deaths), NHL (SMR = 1.17,
27 95% CI = 0.47 to 2.40, 7 observed deaths), multiple myeloma (SMR = 1.84, 95% CI =
28 0.74 to 3.80, 7 observed deaths), and leukemia (SMR = 1.18, 95% CI = 0.54 to 2.24, 9
29 observed deaths). Among workers exposed to styrene and ethylbenzene, there were 16
30 deaths due to lymphohematopoietic malignancies, compared with 8.1 expected [SMR =

1 1.98], and mortality was slightly higher in workers exposed at least 1 year (< 1 year, 4
2 observed vs. 2.6 expected [SMR = 1.54]; \geq 1 year, 12 observed vs. 5.5 expected [SMR =
3 2.2]) and in workers exposed to lower styrene levels (< 5 ppm, 12 observed vs. 5.1
4 expected [SMR = 2.4]; \geq 5 ppm, 4 observed vs. 3.0 expected [SMR = 1.3]). A
5 statistically significant increased risk was found for an analysis that allowed for a 15-year
6 latency period (SMR = 1.60, 95% CI = 1.02 to 2.38, 24 observed deaths), but there was
7 no significant trend of increasing risk with increasing time since first exposure.

8 3.3.3 *United States- single plant*

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

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

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

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

4 *3.3.4 United Kingdom*

5 Hodgson and Jones (1985) studied 622 male workers employed for at least 1 year from
6 1945 to 1974 in a plant where styrene monomer was produced, polymerized, and
7 processed. The workers were followed through 1978, and a total of 8,654 person years
8 were accumulated, with an average follow-up of 13 years. An additional 3,072 male
9 manual workers who had no exposure to styrene but had worked at least one year at the
10 site were identified as a reference group. The lymphomas were confirmed by histological
11 assessment by three pathologists. SMRs were computed from national mortality rates,
12 and standard registration ratios (i.e., SIRs) were computed from regional cancer incidence
13 rates. No measurements of styrene exposure were available, but the authors stated that
14 styrene exposure levels were in general well below 100 ppm. Workers were also exposed
15 to acrylonitrile, and there was potential exposure to benzene, dyestuffs, and ethylene
16 oxide. For the total cohort, the SMRs were 0.90 for all cancer (10 observed vs. 10.9
17 expected), [1.19] for lung cancer (5 vs. 4.2), and [5.36] for lymphoma (3 vs. 0.56, $P =$
18 0.02); no deaths from leukemia were observed (0 vs. 0.3). No excess of deaths from
19 lymphoma or leukemia was observed in the unexposed cohort. The SIRs were 2.50 for all
20 lymphohematopoietic malignancies (4 vs. 1.6, $P = 0.079$), 3.75 for lymphoma (3 vs. 0.8,
21 $P = 0.047$), and [1.67] for leukemia (1 vs. 0.6). An increased incidence of larynx cancer
22 was also reported (3 observed vs. 0.5 expected; P values not given); however no deaths
23 from larynx cancer were reported. The authors stated that laryngeal cancer is often
24 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 <i>et al.</i> 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 <i>Cancers with > 1 death</i> 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 <i>et al.</i> 1980 Bond <i>et al.</i> 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 <i>Total cohort</i> all cancer 0.81 (0.69–0.95), 162 <i>Cancers with non-significant excess mortality</i> 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) <i>Chemical grouping (obs. vs. exp.), LH cancer</i> styrene & ethylbenzene 16 vs. 8.1	Complex mixture of exposures

Study	Population, follow-up, and methods	Exposure	Effects	Comments
Nicholson <i>et al.</i> 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 <i>Air measurements in 1974</i> low exposure: < 1 ppm high exposure: 5–20 ppm	Observed vs. expected deaths <i>Total cohort</i> all cancer 17 vs. 21.01 lung 6 vs. 6.99 leukemia 1 vs. 0.79 lymphoma 1 vs. 1.25 <i>High and low exposure areas</i> 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 <i>Mortality:</i> 1945–78, avg. 13 yr 8,654 person-years <i>Incidence:</i> 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

^aCalculated from the original data using expected deaths from the Rhinehessia-Palatinate region.

1 **3.4 Other cohort studies**

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

3 *3.4.1 Styrene-exposed workers (biomarker study)*

4 Anttila *et al.* identified 2,580 workers (distribution by sex was not reported for the
5 styrene cohort) biomonitored for styrene exposure through measurement of mandelic acid
6 in post-shift urinary samples (Anttila *et al.* 1998). They were followed from the first
7 recorded measurement made between 1973 and 1983, through 1992. A total of 34,288
8 person-years were accumulated [with an average follow-up of 13.3 years]. The median
9 mandelic acid level was 2.3 mmol/L [350 mg/L] (the authors noted that 2.9 mmol/L
10 urinary mandelic acid corresponded roughly to 20 ppm). (Levels were higher in women
11 than men, which the authors stated was probably due to the selection of the monitored
12 task.) Cases of cancer were identified from the Finnish Cancer registry, and SIRs were
13 computed from expected values estimated from cancer incidence rates in the general
14 population. The overall cancer incidence was decreased (SIR = 0.80, 95% CI = 0.59 to
15 1.06, 48 observed cases), and the incidence of rectal cancer was significantly increased
16 (SIR = 3.11, 95% CI = 1.14 to 6.77, 6 observed cases). Increased risks were indicated for
17 cancer of stomach, liver, pancreas, and nervous system and Hodgkin's disease, but none
18 of the findings were statistically significant. When the analysis was limited to workers
19 followed for at least 10 years after first measurement, the SIR was 3.49 (95% CI = 0.72 to
20 10.2, 3 observed cases) for rectal cancer, 3.54 (95% CI = 0.09 to 19.7, 1 observed case)
21 for liver cancer, 3.64 (95% CI = 0.75 to 10.6, 3 observed cases) for pancreatic cancer, and
22 3.11 (95% CI = 0.85 to 7.95, 4 observed cases) for cancer of the nervous system; no cases
23 of lymphohematopoietic malignancy were observed. SIRs were not higher in the high-
24 exposure group (based on lifetime mean urinary metabolite levels) compared with the
25 low-exposure group, but the numbers of observed and expected cases were low. The
26 authors did not provide sex-specific risk estimates but stated that there was no clear
27 difference in the overall incidence pattern between styrene-exposed men and women.

28 *3.4.2 Environmental exposure*

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

1 school adjacent to a styrene-butadiene rubber plant between 1963 and 1993 for at least
2 three consecutive months during a school year. The population was identified from high
3 school yearbooks, and school records (which do not allow recording of sex). Sex was
4 assigned based on data from birth records (73%) of the population, yearbook pictures,
5 and student's name. Data on name changes (such as married name) that occurred after
6 high school were obtained from multiple searches of marriage databases (the first round
7 used the "maiden name," and date of birth, and the second round used the "married
8 name"). The population was followed through 1995, and vital status was obtained from
9 the National Death Index, the Social Security Administration Death Master Files, and the
10 Texas Department of Health death database. Cause of death was obtained from death
11 certificates (matching on maiden or married name, date of birth and state of birth), and
12 SMRs were based on expected numbers calculated from national death rates. A
13 statistically nonsignificant increase in overall cancer mortality was observed for men
14 (SMR = 1.22, 95% CI = 0.83 to 1.73, 31 deaths) but a significant decrease was observed
15 for women (SMR = 0.52, 95% CI = 0.28 to 0.88, 13 deaths). The sex-specific SMRs were
16 as follows (men vs. women): all lymphohematopoietic malignancies, 1.64 (95% CI =
17 0.85 to 2.87, 12 deaths) vs. 0.47 (95% CI = 0.06 to 1.70, 2 deaths); Hodgkin's disease,
18 1.46 (95% CI = 0.18 to 5.28, 2 deaths) vs. 1.20 (95% CI = 0.03 to 6.68, 1 death); and
19 leukemia, 1.82 (95% CI = 0.67 to 3.96, 6 deaths) vs. 0.45 (95% CI = 0.01 to 2.48, 1
20 death). Among males, the SMR's for subtypes of lymphohematopoietic cancers were
21 somewhat higher in those who attended school for 2 years or less compared with those
22 who attended more than 3 years. [Note that only the SMR for leukemia+aleukemia
23 among those attending high school for < 2 years was significantly elevated, SMR = 5.29,
24 (95% CI = 1.09 to 15.46, 3 deaths)]. A significant excess of deaths from benign
25 neoplasms (all of which were brain tumors) also was observed in men (SMR = 6.27, 95%
26 CI = 2.04 to 14.63, 5 deaths); only one case was observed in females (SMR = 1.56, 95%
27 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 <i>et al.</i> 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 <i>Total cohort</i> all cancer 0.80 (0.59–1.06), 48 <i>Cancers with significantly or nonsignificantly increased incidences</i> 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 <i>≥ 10 years after first measurement</i> <i>Cancers with increased incidences^a</i> 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 <i>et al.</i> 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 <i>Men</i> (241 deaths) <i>Cancers with excess mortality (significant or non-significant)</i> 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 <i>Women</i> (97 deaths) all cancer 0.52 (0.28–0.88), 13 <i>Cancers with non-significant excess mortality</i> 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

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

1 **3.5 Case-control and ecological studies**

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

6 *3.5.1 Lymphohematopoietic cancers*

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

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

1 Seidler *et al.* (2007) conducted a population-based, case-control study of exposure to
2 chlorinated and aromatic organic solvents and malignant lymphoma incidence among
3 men and women between 18 and 80 years of age in six regions of Germany. Cases (710)
4 were matched by age, region, and gender to equal numbers of population controls.
5 Cumulative occupational exposures were evaluated by detailed personal work histories
6 obtained by face-to-face questionnaire and assessment by an occupational physician;
7 exposure was estimated by both duration (% of work time) and 3 levels of exposure. Data
8 were analyzed by conditional logistic regression. In comparison with 542 cases with no
9 estimated exposure to styrene, the ORs for malignant lymphoma associated with styrene
10 exposure, after adjustment for smoking and alcohol consumption, were 0.7 (95% CI = 0.5
11 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
12 67.1 ppm-years; and 0.8 (95% CI = 0.3 to 1.4, 12 cases) for > 67.1 ppm-years. No
13 significant trend with exposure was observed ($P = 0.43$). No elevated risks were observed
14 when lymphoma subtypes were considered. No attempt was made in this study to adjust
15 for potential confounding by multiple exposures, including other aromatic hydrocarbons
16 and chlorinated hydrocarbons. [Note that chlorinated hydrocarbons, but not other
17 aromatic hydrocarbons, were associated with an elevated risk of malignant lymphoma.]

18 3.5.2 *Breast cancer*

19 Cantor *et al.* (1995) conducted a population-based, case-control study of breast cancer
20 mortality based on records gathered between 1984 and 1989 from 24 U.S. states. Cases
21 were 33,509 women with breast cancer listed as the underlying cause of death on the
22 death certificate, and 117,794 controls were randomly selected from non-cancer deaths
23 matched for gender, age within 5 years, and race. Homemakers were excluded from the
24 analysis. A JEM was used to classify cases and controls with respect to specific
25 occupational exposures and the probability and level of exposure. The JEM was based on
26 the usual occupation and industry listed on the death certificate, from which an industrial
27 hygienist assigned a probability of exposure or a probable level of exposure for a total of
28 31 agents or groups of agents. Analyses were adjusted for age at death and, in some
29 cases, socioeconomic status, and results were presented separately for black and white
30 women. Among white women, in comparison with 27,610 cases with no estimated

1 styrene exposure, the ORs for breast cancer, adjusted for age and socioeconomic status,
2 were 1.16 (95% CI = 1.1 to 1.3, 807 cases) for low exposure, 1.13 (95% CI = 1.0 to 1.3,
3 522 cases) for medium exposure and 1.19 (95% CI = 0.9 to 1.6, 70 cases) for the highest
4 exposure category. Among black women, in comparison with 3,918 non-exposed cases,
5 the adjusted ORs for low- and medium-exposure levels were 1.59 (95% CI = 1.2 to 2.1,
6 87 cases) and 1.41 (95% CI = 1.0 to 1.9, 63 cases), respectively. Thus, breast cancer
7 showed a weak but statistically significant association with styrene exposure, with ORs
8 generally about 1.2 for whites and 1.5 for blacks. No clear trend by exposure probability
9 or exposure level was seen that was consistent across the two races. [Note that no other
10 exposures investigated were significantly associated with breast cancer in this population,
11 with the exception of a weak association with asbestos and non-ionizing radiofrequency
12 and ionizing radiation.]

13 Coyle *et al.* (2005) conducted an ecological study to evaluate the relationship between
14 invasive breast cancer incidence and releases of 12 selected environmental toxicants
15 reported in the paper to be associated with breast cancer (the chemicals carbon
16 tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, and
17 trichloroethylene and the metals arsenic, cadmium, chromium, cobalt, copper, and nickel)
18 that were reported to the Environmental Protection Agency as being released in one or
19 more of 254 counties in Texas during 1988 to 2000. During the years 1995 through 2000,
20 54,487 cases of breast cancer (in both men and women) were identified from the Texas
21 Cancer Registry. For each toxicant, the age-adjusted breast cancer rate for each of these
22 counties was compared with the amount of toxicant released in that county, based on
23 information obtained from the EPA Toxics Release Inventory (TRI) for 1988 to 2000. In
24 a univariate analysis, the median age-adjusted annual breast cancer incidence was
25 significantly higher in counties reporting releases of styrene (and several other
26 compounds) than counties not reporting releases (66.2 cases in 61 counties vs. 59.8 cases
27 in 193 counties, respectively, $P < 0.001$). A multivariate analysis model of breast cancer
28 and exposure to the environmental toxicants (that were significantly associated with
29 breast cancer in the univariate analyses) found significant positive associations between
30 release of styrene and breast cancer in women and men ($P = 0.0004$), women ($P = 0.002$),
31 and women aged 50 or older ($P = 0.002$). [No other data were presented, and it is not

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

8 3.5.3 Series of studies in a Canadian population

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

25 In analyses that focused on four different organic solvents, Gerin *et al.* (1998) found
26 statistically significant increased ORs with respect to exposure to medium/high levels of
27 styrene for rectal cancer (OR = 5.1, 95% CI = 1.4 to 19.4, 5 cases), and prostate cancer
28 (OR = 5.5, 95% CI = 1.4 to 21.8, 7 cases), and statistically nonsignificant increased risks
29 of NHL (OR = 2.0, 95% CI = 0.8 to 4.8, 8 cases), and Hodgkin's lymphoma (OR = 2.4,
30 95% CI = 0.5 to 11.6, 2 cases). No increases in the risk for cancer of the esophagus,

1 stomach, colon, pancreas, lung, bladder, or kidney or melanoma of the skin were
2 observed. [Note that these ORs were adjusted for demographic and socioeconomic
3 covariates, smoking, and respondent status but not for other exposures.] Only 2% of the
4 population was classified as exposed to styrene and only 45% of those were considered to
5 be “certainly” exposed. Dumas *et al.* (2000) focused on a broad spectrum of occupational
6 factors and the risk of rectal cancer and found a statistically increased unadjusted OR for
7 styrene exposure (for “substantial exposure,” unadjusted OR = 3.9, 95% CI = 1.2 to 12.9,
8 5 cases; for “any” exposure, OR adjusted for demographic and lifestyle factors = 1.7,
9 95% CI = 0.7 to 4.5, 6 cases. Note that no adjustment for potential confounders was
10 conducted for the “substantial” exposure group). Parent *et al.* (2000) examined the risk
11 factors for renal-cell carcinoma and found that the OR for exposure to styrene-butadiene
12 rubber was significantly increased for renal-cell carcinoma among the “any exposure”
13 group (OR = 2.1, 95% CI = 1.1 to 4.2, 10 cases, adjusted for demographic and lifestyle
14 variables, but the risk was somewhat attenuated when additionally adjusted for felt dust
15 exposure (OR = 1.8, 95% CI = 0.9 to 3.7).

16 3.5.4 Lung cancer and styrene exposure

17 Scélo *et al.* (2004) conducted a clinic-based, case-control study of 2,861 patients (of a
18 total of 3,403) diagnosed with lung cancer between 1998 and 2002 in Romania, Hungary,
19 Poland, Russia, Slovakia, the Czech Republic, and the United Kingdom. There were
20 3,118 hospital or population controls. Cases and controls were interviewed about the
21 characteristics of each job held, and chemists and hygienists translated each job case-by-
22 case into potential exposure to styrene and 70 other agents. The OR for lung cancer
23 among patients ever exposed to styrene (N = 51) was 0.7 (95% CI = 0.42 to 1.18;
24 adjusted for center, gender, age, smoking, vinyl chloride, acrylonitrile, formaldehyde, and
25 inorganic pigments). No trends with duration of exposure or cumulative exposure were
26 observed.

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

Study	Population and methods	Exposure	Effects	Comments				
Flodin <i>et al.</i> 1986 Sweden	<p>clinic-based case-control study</p> <p><i>Cases:</i> 59 Swedish patients (aged 20–70) diagnosed with acute myeloid leukemia at medical clinics or departments in hospitals, 1977–82</p> <p><i>Controls:</i> 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</p> <p>ORs calculated by logistic regression</p>	Exposure to styrene and other agents (radiation, solvents), and information on smoking and other lifestyle exposures assessed by questionnaire	<p>OR (95% CI), cases/controls (unadjusted)</p> <p><i>Styrene exposure</i></p> <p>acute myeloid leukemia 18.9 (1.9–357), 3/1</p>	<p>Self-reported exposure information obtained after diagnosis was made</p> <p>Only 3 styrene-exposed cases</p>				
Guenel <i>et al.</i> 2002 France	<p>nested case-control study of active utility workers</p> <p><i>Cases:</i> 72 cases of leukemia diagnosed 1978–89 (< 60 years old)</p> <p><i>Controls:</i> 285 controls (4 per case) matched on birth year</p> <p>ORs calculated by conditional logistic regression</p>	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	<p>OR (95% CI), no. of cases/controls</p> <p><i>Styrene exposure</i></p> <p>Leukemia OR (adjusted for benzene + other chemicals) 1.1 (0.2–5.9), 2/9</p>					
Cantor <i>et al.</i> 1995 U.S.	<p>population-based, case-control study</p> <p><i>Cases:</i> 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</p> <p><i>Controls:</i> 117,794 controls (4/case)</p>	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	<p>adjusted OR (95% CI), no. of cases adjusted for age at death and socioeconomic status</p> <p><i>Styrene exposure and breast cancer</i></p> <p><i>White women</i></p> <p>probability</p> <table border="0"> <tr> <td>1</td> <td>1.13 (1.0–1.2), 804</td> </tr> <tr> <td>2</td> <td>1.18 (1.1–1.3), 527</td> </tr> </table>	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
1	1.13 (1.0–1.2), 804							
2	1.18 (1.1–1.3), 527							

Study	Population and methods	Exposure	Effects	Comments
	<p>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</p> <p>ORs adjusted for age at death and socioeconomic status</p>	<p>agents, including styrene</p>	<p>3 1.38 (1.0–1.9), 64 4 NR</p> <p>level</p> <p>1 1.16 (1.1–1.3), 807 2 1.13 (1.0–1.3), 522 3 1.19 (0.9–1.6), 70</p> <p><i>Black women</i></p> <p>probability</p> <p>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</p> <p>level</p> <p>1 1.59 (1.2–2.1), 87 2 1.41 (1.0–1.9), 63 3 NR</p>	
<p>Coyle <i>et al.</i> 2005 Texas, USA</p>	<p>ecological study</p> <p>54,487 cases of invasive breast cancer in men and women reported to the Texas Cancer Registry, 1995–2000</p> <p>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</p> <p><i>Univariate analysis:</i> Mann-Whitney U test used to compare median average annual age-adjusted breast cancer rates in counties reporting releases vs. those not reporting releases</p> <p>Stepwise multiple linear regression models included age, race, ethnicity, and toxicants</p>	<p>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.</p> <p>Toxicants were chosen based on (1) reporting of an association with breast cancer</p>	<p><i>Univariate analysis</i></p> <p>median average annual age-adjusted breast cancer rate in Texas counties</p> <p><i>Reported release of styrene</i></p> <p>yes 66.2 cases no 59.8 cases, $P < 0.001$</p> <p><i>Multiple linear regression: styrene and age-adjusted breast cancer rate</i></p> <p>β, P, explained variance (%)</p> <p>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</p>	<p>Criterion for exposure (≥ 1 releases reported in TRI in a given county) unlikely to reflect individual styrene exposure</p> <p>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</p>

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 <i>et al.</i> 1998 ^a Dumas <i>et al.</i> 2000 Parent <i>et al.</i> 2000 Canada	<p>population-based case-control study</p> <p>Cases: 3,730 men aged 35–70 with cancer identified 1979–86; cancers at 15 sites studied^a</p> <table border="1"> <thead> <tr> <th>Cancer</th> <th>no. of cases</th> </tr> </thead> <tbody> <tr> <td>renal-cell carcinoma</td> <td>142</td> </tr> <tr> <td>rectal</td> <td>257</td> </tr> <tr> <td>prostate</td> <td>449</td> </tr> <tr> <td>NHL</td> <td>215</td> </tr> <tr> <td>Hodgkin’s disease</td> <td>54</td> </tr> </tbody> </table> <p>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 <i>et al.</i> used the 533 population controls and a subset (533) of the cancer controls</p> <p>ORs computed by unconditional logistic regression analysis with adjustment for age, respondent status, cigarette smoking (all publications), plus (1) family income, ethnic group (Gérin <i>et al.</i>) (2) education, beer consumption, body mass index (Dumas <i>et al.</i>)</p>	Cancer	no. of cases	renal-cell carcinoma	142	rectal	257	prostate	449	NHL	215	Hodgkin’s disease	54	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	<p><i>Styrene exposure and various cancers, using pooled (population & cancer) controls</i> (Gérin <i>et al.</i>) adjusted OR (95% CI), cases/controls</p> <p><i>Cancers with increased ORs</i></p> <p><i>Medium or high exposure</i></p> <table border="1"> <tbody> <tr> <td>rectum</td> <td>5.1 (1.4–19.4), 5/4</td> </tr> <tr> <td>prostate</td> <td>5.5 (1.4–21.8), 7/3</td> </tr> <tr> <td>esophagus</td> <td>1.4 (0.5–3.8), 5/40</td> </tr> </tbody> </table> <p><i>Ever exposed</i></p> <table border="1"> <tbody> <tr> <td>NHL</td> <td>2.0 (0.8–4.8), 8/19</td> </tr> <tr> <td>Hodgkin’s disease</td> <td>2.4 (0.5–11.6), 2/19</td> </tr> </tbody> </table> <p><i>Styrene exposure and rectal cancer, using cancer controls</i> (Dumas <i>et al.</i>) adjusted OR (95% CI), no. of exposed cases</p> <table border="1"> <tbody> <tr> <td>any</td> <td>1.7 (0.7–4.5), 6</td> </tr> <tr> <td>substantial</td> <td>3.9 (1.2–12.9), 5</td> </tr> </tbody> </table> <p><i>Styrene-butadiene rubber exposure and renal-cell cancer, using pooled controls</i> (Parent <i>et al.</i>) adjusted OR (95 % CI), no. of exposed cases</p> <table border="1"> <tbody> <tr> <td>Model 1</td> <td>2.1 (1.1–4.2), 10</td> </tr> <tr> <td>Model 2</td> <td>1.8 (0.9–3.7), 10</td> </tr> </tbody> </table>	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	NHL	2.0 (0.8–4.8), 8/19	Hodgkin’s disease	2.4 (0.5–11.6), 2/19	any	1.7 (0.7–4.5), 6	substantial	3.9 (1.2–12.9), 5	Model 1	2.1 (1.1–4.2), 10	Model 2	1.8 (0.9–3.7), 10	<p>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</p> <p>Only 2% of the population had potential styrene exposure</p>
Cancer	no. of cases																																	
renal-cell carcinoma	142																																	
rectal	257																																	
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Hodgkin’s disease	54																																	
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Model 1	2.1 (1.1–4.2), 10																																	
Model 2	1.8 (0.9–3.7), 10																																	

Study	Population and methods	Exposure	Effects	Comments
	(3) body mass index (Parent <i>et al.</i>): Model 1 (4) body mass index and felt dust exposure (Parent <i>et al.</i>): Model 2			
Scélo <i>et al.</i> 2004 Romania, Hungary, Poland, Russia, Slovakia, Czech Republic, U.K.	<p>clinic-based, case-control study of lung cancer</p> <p><i>Cases:</i> 2,861 patients with newly diagnosed lung cancer that occurred 1998–2002; patients recruited from 15 hospital centers</p> <p><i>Controls:</i> 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.</p> <p>ORs calculated by unconditional logistic regression and adjusted for age, gender, center, tobacco consumption, and exposure to occupational agents</p>	<p>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</p> <p>0.6% of controls had potential exposure to styrene based on jobs held</p>	<p>Adjusted OR (95% CI), cases/controls</p> <p><i>Styrene exposure and lung cancer</i></p> <p>Ever exposed 0.70 (0.42–1.18), 51/47 (adjusted for age, gender, center, smoking, vinyl chloride, acrylonitrile, formaldehyde, organic pigments)</p> <p>Risk of lung cancer did not increase with increasing duration of exposure, weighted duration of exposure, or cumulative exposure</p>	<p>80% statistical power to detect OR for ever exposure in the range of 1.5 to 1.6</p>

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

CI = confidence interval, NHL = non-Hodgkin’s lymphoma, NR = not reported, OR = odds ratio.

^aGerin *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.

1 **3.6 [Strengths and limitations of the literature]³**

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

5 *3.6.1 Utility of the studies*

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

18 On the other hand, studies of the reinforced-plastics industry included few long-term
19 workers, with the exception of the large multi-country cohort of Kogevinas *et al.* (1994a,
20 1993). Of approximately 85,000 reinforced-plastics workers studied, the majority were
21 employed for less than one year, and fewer than 7,500 were employed for more than 10
22 years. An estimated 40% of the latter workers were laminators (the workers with the
23 highest styrene exposure level), so the database includes results for only 3,000 long-term
24 workers exposed to styrene at high levels. Secondly, the average follow-up was less than
25 15 years for three of the most informative populations (Anttila *et al.* 1998, Kogevinas *et*
26 *al.* 1994a, Kolstad *et al.* 1995). Thirdly, as in the case of the majority of other populations
27 exposed to styrene, exposures have been considerably reduced over the past decades (for
28 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.

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

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

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

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

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

1 Montreal-based study were considered to be potentially exposed to any level of styrene).
2 The one occupational case-control study, of utility workers by Guenel *et al.* (2002), was
3 primarily focused on electromagnetic field exposure and yielded only 11 of 357 workers
4 (3%) with potential styrene exposure. In addition, the general lack of precision with
5 which exposures were assigned also reduced the power of these studies to detect an
6 effect, as discussed below. However, in the case of the large case-control study of breast
7 cancer (Cantor *et al.* 1995), this study may be of value in assessing the risk of this cancer
8 among the women exposed to styrene, since there is generally insufficient power to detect
9 breast cancer risk among women in cohort studies due to the small number of exposed
10 women.

11 3.6.2 *Misclassification of disease and exposure*

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

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

1 Sathiakumar *et al.* 2005). (AML, CML, and adult ALL arise from the same pluripotential
2 stem cell, based on observations of specific genetic re-arrangements in these 3 subtypes
3 of leukemia, which comprise about 80% of adult leukemias. For example, the blast crisis
4 of CML, 90% of which have the Philadelphia chromosome, cannot be distinguished from
5 AML. An estimated 10% of adult ALL cases have the Philadelphia chromosome, which
6 suggests a common stem-cell origin for these leukemias (Bloomfield *et al.* 1978, Jacobs
7 1989, Yunis 1983). There is considerable overlap between CLL and NHL; CLL and NHL
8 (85%) are B-cell malignancies (Delzell *et al.* 2006) and CLL is the same disease as small
9 lymphocytic lymphoma (Delzell *et al.* 2006, Harris *et al.* 2000). Delzell *et al.* 2006
10 grouped NHL+CLL in their data analyses.)

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

27 In several of the case-control studies, reliance is placed on self-administered or in-person
28 questions to establish either jobs held or potential exposures among living respondents
29 combined with assignation of exposure by a member of the research team (Dumas *et al.*
30 2000, Flodin *et al.* 1986, Gérin *et al.* 1998, Parent *et al.* 2000, Scélo *et al.* 2004, Seidler *et*

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

10 Classification of workers by individual job titles (McMichael *et al.* 1976a, Ruder *et al.*
11 2004) or job-exposure matrices (Bond *et al.* 1992, Delzell *et al.* 2001, Kogevinas *et al.*
12 1994a, Matanoski *et al.* 1997, Santos-Burgoa *et al.* 1992, Seidler *et al.* 2007, Wong *et al.*
13 1994) may, at least partly, have reduced misclassification of exposure. [Exposure
14 classification by job-exposure matrices is preferable since workers may experience
15 different exposures within given departments according to the particular job performed
16 and may move between one job and another within and across departments.] However, in
17 a validation test within the styrene-butadiene rubber industry, styrene exposure ranks
18 correlated poorly with styrene measurements (Matanoski *et al.* 1993), clearly illustrating
19 that it may be difficult to obtain valid exposure estimates for styrene in this industry. If
20 exposure ranks and actual measurements correlate poorly, this would tend to attenuate
21 any apparent risk and bias the findings towards the null. Macaluso *et al.* (2004) generally
22 found estimates of styrene exposure in the styrene-butadiene rubber industry to be lower
23 than industrial hygiene measurements but did not conduct a thorough validation of their
24 exposure estimates.

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

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

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

10 Such misclassification of styrene exposure was independent of health outcome and thus
11 would be expected to be nondifferential and to bias any measures of association towards
12 no effect. Exceptions were (1) the studies reported by Delzell and colleagues using the
13 revised exposure assessment (Delzell *et al.* 2001, Graff *et al.* 2005, Delzell *et al.* 2006),
14 because the investigators were aware of the employment histories of the workers who had
15 died of leukemia when they revised their exposure estimates, and (2) the population or
16 clinic-based, case-control studies, because they relied on patients' retrospective
17 descriptions of exposures or working conditions (Dumas *et al.* 2000, Flodin *et al.* 1986,
18 Gérin *et al.* 1998, Parent *et al.* 2000, Scélo *et al.* 2004, Seidler *et al.* 2007). Kolstad *et al.*
19 (1994) compared exposure data obtained from employers in the reinforced-plastics
20 industry with those obtained from dealers in raw materials and found indications that
21 employers' reports were not independent of health outcome for some companies; the
22 employers' reports therefore were omitted from the analyses.

23 3.6.3 *Other possible biases and confounding*

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

1 of correlation between exposures is observed, such as in the styrene-butadiene industry.
2 Several studies adjusted for butadiene (Delzell *et al.* 2001, Delzell *et al.* 2006, Graff *et al.*
3 2005, Santos-Burgoa *et al.* 1992) and DMDTC (Delzell *et al.* 2001, Delzell *et al.* 2006,
4 Graff *et al.* 2005). Graff *et al.* (2005) noted that styrene, butadiene, and DMDTC
5 exposure are highly correlated, and it is difficult to separate the effects of one agent from
6 the other two agents. Butadiene is classified as a known human carcinogen by IARC and
7 the NTP, and is considered to be a risk factor for leukemia (IARC 1999, NTP 2004).
8 DMDTC is less strongly correlated with styrene exposure than butadiene, 0.6 compared
9 with 0.8 (Delzell *et al.* 2001). It is considered to be an immune system depressant (T-cell)
10 (Delzell *et al.* 2006), but its carcinogenicity has not been evaluated outside the studies in
11 the styrene-butadiene industry. Although there is potential exposure to benzene in the
12 styrene-butadiene rubber industry, it was not considered to have an impact on leukemia
13 based on studies by Macaluso *et al.* 1996 (Delzell *et al.* 2006).

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

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

1 1990). Certain lifestyle factors and/or other occupational factors were included in
2 analyses of the clinic- and population-based, case-control studies (Dumas *et al.* 2000,
3 Flodin *et al.* 1986, Gérin *et al.* 1998, Parent *et al.* 2000, Scélo *et al.* 2004, Seidler *et al.*
4 2007), but not in the cohort-based studies. Internal analyses within the worker
5 populations are expected to be less sensitive to confounding by lifestyle factors, because
6 the populations are expected to be relatively homogenous with respect to socioeconomic
7 factors (Delzell *et al.* 2001, Kogevinas *et al.* 1994a, Kolstad *et al.* 1995, Kolstad *et al.*
8 1994, Wong *et al.* 1994). However, confounding cannot be ruled out, because little is
9 known about the causes of the majority of the malignant diseases studied.

10 Short-term workers in the reinforced-plastics industry showed generally higher cancer
11 risk than long-term workers (Kogevinas *et al.* 1994a, 1995, Kolstad *et al.* 1994, Ruder *et*
12 *al.* 2004, Wong *et al.* 1994). This might be because short-term workers are mainly
13 assigned to jobs with high styrene exposure; however, no data are available to assess this
14 hypothesis. The finding might also be explained by the healthy-worker effect — that is, a
15 selection process by which workers who become unfit during employment tend to leave.
16 However, the healthy-worker effect is generally less for malignant diseases than for
17 chronic nonmalignant diseases (Arrighi and Hertz-Picciotto 1994). A third explanation
18 might be confounding because of differences in other risk factors between short- and
19 long-term workers. A separate study supported this hypothesis; it showed that Danish
20 short-term reinforced-plastics workers had been hospitalized for lifestyle-related health
21 conditions before employment in the industry more often than long-term workers
22 (Kolstad and Olsen 1999). Thus, confounding by factors related to lifestyle is a likely
23 explanation, at least to some extent, of the unexpected decline in risk with length of
24 employment. One way of handling such confounding would be by comparison with a
25 non-styrene-exposed group of short-term workers with expected comparable
26 socioeconomic status and lifestyle factors. Such analysis of the Danish reinforced-plastics
27 industry workers showed a statistically nonsignificant increased risk of leukemia (RR =
28 1.89, 95% CI = 0.78 to 4.59) (Kolstad *et al.* 1994).

1 **3.7 Summary of previous evaluations (IARC and Cohen *et al.*)**

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

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

12 IARC (2002) regarded data from the reinforced-plastics industry as the most informative,
13 because workers in that industry were exposed to the highest levels of styrene and had
14 less potential for exposure to other substances within the occupational setting than the
15 other cohorts studied. The IARC evaluation emphasized a small, nonsignificantly
16 increased incidence of leukemia among Danish reinforced-plastics workers and a
17 statistically significant excess among those workers with the earliest first years of
18 employment, the highest styrene exposure levels, or latency of at least 10 years.

19 However, among all workers exposed for 1 year or more, the incidence of leukemia was
20 not increased. In a European multinational cohort of reinforced-plastics workers (that
21 partially overlapped with the Danish study), mortality from lymphatic and hematopoietic
22 neoplasms was not increased, based on comparison with national reference rates.

23 However, in an internal analysis using the unexposed workers as the comparison group,
24 mortality was increased in exposed workers after 20 years since the first exposure to
25 styrene and also increased with increasing intensity of exposure, but not with increasing
26 cumulative exposure to styrene. A large U.S. mortality study of reinforced-plastics
27 workers found no overall excess of lymphohematopoietic malignancies. IARC stated that
28 problems with mortality ascertainment in the European study and underestimation of
29 duration of exposure in the Danish study might have influenced the findings.

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

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

28 **3.8 Summary of the findings for selected cancer sites**

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

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

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

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

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

1 the male Danish workers (results were not presented for female workers [(Kolstad *et al.*
2 1995, Kolstad *et al.* 1994)] were omitted from the dataset of the European reinforced-
3 plastics industry (Kogevinas *et al.* 1994a). This was done based on country-specific
4 findings published in an IARC report describing details of the European study
5 (Kogevinas *et al.* 1994b). [This was done to eliminate overlap between the Danish and
6 the European datasets and thus erroneously pooled estimates. Another option would be to
7 exclude the total Danish dataset (as Cohen *et al.* 2002 did in their review), but this would
8 mean that the results based on that part of the Danish population not included in the
9 European data set would be left out. Furthermore, the Danish study reported incidence
10 data while the European study only reported mortality data, and incidence data is
11 regarded the most relevant for several of the cancers studied with relatively low
12 mortality. Therefore, inclusion of the Danish population and exclusion of the male
13 Danish workers from the European study when pooling observed and expected number of
14 cases, is expected to have improved the validity of the overall evaluation.]

15 [The data in Tables 3-8 to 3-10 permit a general comparison of multiple studies and the
16 identification of trends in the data (e.g., several studies reporting nonsignificant increases
17 in a specific site). However, there are several limitations inherent in pooling data across
18 different studies. Study populations may differ with respect to, for example, the size and
19 type of population studied, their racial or age composition, inclusion and exclusion
20 criteria (such as minimum duration of employment or exposure time), latency periods,
21 duration of follow-up, the nature and intensity of exposure, and the type of cases reported
22 (incidence vs. mortality). In addition, studies clearly vary in quality, e.g., with respect to
23 the power of the study, (especially for specific cancer sites) and which, if any, potentially
24 confounding variables (e.g., potential exposure to other carcinogens) are adjusted for.
25 This methodology also does not incorporate information on subgroup analyses (such as
26 exposure-response relations) that may be important in evaluating causality. However, as
27 mentioned previously, one of the major limitations of the body of literature is the small
28 numbers of highly exposed workers, which limits the ability to detect an effect, especially
29 for uncommon tumors. This approach (summing observed and expected cases of highly
30 exposed workers from all studies) facilitates the evaluation of the relationship between
31 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.	
	Ruder <i>et al.</i> 2004	Wong <i>et al.</i> 1994	Kolstad <i>et al.</i> 1994, 1995	Kogevinas <i>et al.</i> 1994 ^a	Anttila <i>et al.</i> 1998	McMichael <i>et al.</i> 1976	Sathia-kumar <i>et al.</i> 2005 ^c	Frentzel-Beyme <i>et al.</i> 1978	Bond <i>et al.</i> 1992	Nicholson <i>et al.</i> 1978	Hodgson and Jones 1985		Loughlin <i>et al.</i> 1999	
							Delzell <i>et al.</i> 2006				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.		
	Ruder <i>et al.</i> 2004	Wong <i>et al.</i> 1994	Kolstad <i>et al.</i> 1994, 1995	Kogevinas <i>et al.</i> 1994 ^a	Anttila <i>et al.</i> 1998	McMichael <i>et al.</i> 1976	Sathiakumar <i>et al.</i> 2005 ^c	Frentzel-Beyme <i>et al.</i> 1978	Bond <i>et al.</i> 1992	Nicholson <i>et al.</i> 1978	Hodgson and Jones 1985		Loughlin <i>et al.</i> 1999	
											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.	
	Ruder <i>et al.</i> 2004	Wong <i>et al.</i> 1994	Kolstad <i>et al.</i> 1994, 1995	Kogevinas <i>et al.</i> 1994 ^a	Anttila <i>et al.</i> 1998	McMichael <i>et al.</i> 1976	Sathiakumar <i>et al.</i> 2005 ^c	Frentzel-Beyme <i>et al.</i> 1978	Bond <i>et al.</i> 1992	Nicholson <i>et al.</i> 1978	Hodgson and Jones 1985		Loughlin <i>et al.</i> 1999	
			Delzell <i>et al.</i> 2006	SMR			SIR				M	F		
pleura			(+)											
mediastinum			(+)											
breast	(-)	(-)		-	(-)									(-)
female genital organs	(+)	+ ^c			(+)									
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.	
	Ruder <i>et al.</i> 2004	Wong <i>et al.</i> 1994	Kolstad <i>et al.</i> 1994, 1995	Kogevinas <i>et al.</i> 1994 ^a	Anttila <i>et al.</i> 1998	McMichael <i>et al.</i> 1976	Sathiakumar <i>et al.</i> 2005 ^c	Frentzel-Beyme <i>et al.</i> 1978	Bond <i>et al.</i> 1992	Nicholson <i>et al.</i> 1978	Hodgson and Jones 1985		Loughlin <i>et al.</i> 1999	
			Delzell <i>et al.</i> 2006	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.	
	Ruder <i>et al.</i> 2004	Wong <i>et al.</i> 1994	Kolstad <i>et al.</i> 1994, 1995	Kogevinas <i>et al.</i> 1994 ^a	Anttila <i>et al.</i> 1998	McMichael <i>et al.</i> 1976	Sathiakumar <i>et al.</i> 2005 ^c	Frentzel-Beyme <i>et al.</i> 1978	Bond <i>et al.</i> 1992	Nicholson <i>et al.</i> 1978	Hodgson and Jones 1985		Loughlin <i>et al.</i> 1999	
							Delzell <i>et al.</i> 2006				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.

1 3.8.1 Esophageal cancer

2 *Reinforced plastic workers*: Statistically significantly increased risks were observed in the
3 two U.S. studies (Ruder *et al.* 2004 and Wong *et al.* 1994), and a nonsignificantly
4 increased risk was observed for laminators, but not for workers with unspecified tasks or
5 other exposed jobs, in the European cohort (Kogevinas *et al.* 1994a). The SIR in the
6 Danish study was close to unity (Kolstad *et al.* 1995). Among the worker categories with
7 the highest potential styrene exposure — laminators (Kogevinas *et al.* 1994a, Ruder *et al.*
8 2004), open-process mold workers (Wong *et al.* 1994), and workers at companies
9 employing 50% to 100% laminators (Kolstad *et al.* 1995) – a total of 14 cases of
10 esophageal cancer were observed (≥ 7.2 expected⁴; see Table 3-10). A statistically
11 nonsignificant trend toward increased esophageal cancer mortality with increasing
12 cumulative styrene exposure was seen among European reinforced-plastics workers
13 (Kogevinas *et al.* 1994a), and mortality was highest at ≥ 20 years since first exposure.
14 Mortality was increased (SMR = 2.74, 95% CI = 0.004 to 22.3, 1 observed death) among
15 workers from Washington state with high exposure for more than 1 year (Ruder *et al.*
16 2004).

17 *Other industries*: Among workers in the styrene-butadiene rubber industry, the SMR for
18 esophageal cancer was close to unity (SMR = 0.94, 95% CI = 0.68 to 1.26, 44 observed
19 deaths) (Sathiakumar *et al.* 1998). Among styrene monomer production workers, 4 cases
20 were identified, compared with 5.1 expected (Bond *et al.* 1992, Hodgson and Jones
21 1985). [Evaluation of site-specific cancer risks in this industry is limited by the small
22 numbers of subjects; three of the four studies had low numbers (fewer than 20) of
23 expected and observed cases of all malignant tumors.] Risk estimates for esophageal
24 cancer were not reported for the biomonitoring study (Anttila *et al.* 1998) or the study of
25 environmental exposure to styrene-butadiene (Loughlin *et al.* 1999).

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

⁴ “ \geq ” is used when at least one study reported the number of observed cases but not expected cases.

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

4 3.8.2 Pancreatic cancer

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

18 *Other industries*: In styrene-butadiene rubber industry workers, the SMR was 0.87 (95%
19 CI = 0.68 to 1.08; 76 observed deaths) (Sathiakumar *et al.* 1998). The findings from the
20 styrene monomer and polymer industry were divergent; decreased mortality (non-
21 significant) was reported by Bond *et al.* (1992) and an increased mortality (non-
22 significant) was reported by Frentzel-Beyme *et al.* (1978) and the pooled number of cases
23 was less than expected (7 observed vs. 11 expected). The biomonitored workers (Anttila
24 *et al.* 1998) showed a 3-fold increased risk of pancreatic cancer (SIR = 3.64; 95% CI =
25 0.75 to 10.6, 3 cases) 10 years or more after the first measurement. No risk estimate was
26 reported in the environmental exposure study.

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

1 3.8.3 Laryngeal cancer

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

6 *Other industries:* The SMR value was non-significantly decreased (SMR = 0.71, 95% CI
7 = 0.41 to 1.13, 17 observed deaths) in the styrene-butadiene rubber industry (Sathiakumar
8 *et al.* 2005). In the styrene monomer and polymer industry, only 1 case (death) was
9 reported (vs. 2.9 expected) (Bond *et al.* 1992). Hodgson and Jones (1985) reported an
10 excess of incidence cases (3 observed vs. 0.5 expected, $P = 0.041$); however, no mortality
11 was reported. The authors stated that laryngeal cancer is often amenable to treatment.
12 Risk estimates were not calculated in the biomonitoring or environmental studies, and
13 there were no case-control studies evaluating laryngeal cancer.

14 3.8.4 Lung cancer

15 *Reinforced-plastics workers:* Lung cancer risk was significantly increased among U.S.
16 workers (Wong *et al.* 1994), and increased but not statistically significant among workers
17 from Denmark (Kolstad *et al.* 1995) and Washington state (Ruder *et al.* 2004). Among
18 the highest-styrene-exposure group in the reinforced-plastics industry, 158 cases of lung
19 cancer were observed, compared with 151.5 expected (Table 3-10). Lung cancer risk was
20 lower among styrene-exposed workers in a nested case-control study that controlled for
21 smoking, in long-term workers, and among workers with higher cumulative styrene
22 exposure (Kogevinas *et al.* 1994a, Kolstad *et al.* 1995, Ruder *et al.* 2004, Wong *et al.*
23 1994).

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

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

4 3.8.5 Lymphohematopoietic cancers

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

9 3.8.5.1 All lymphohematopoietic cancers combined

10 *Reinforced-plastics workers:* Kolstad *et al.* 1994 reported a non-significantly increased
11 incidence for all lymphohematopoietic malignancies (SIR = 1.20; 95% CI = 0.98 to 1.44,
12 112 observed cases) among Danish workers (which overlaps with the international study
13 reported by Kogevinas *et al.* (1994a, 1993). No increase in lymphohematopoietic cancer
14 mortality was observed for the two U.S. studies (Ruder *et al.* 2004, and Wong *et al.*
15 1994). Among all workers in the reinforced plastic industry, 196 cases were observed
16 compared with 199.2 expected (Table 3-9). Observed among the high-styrene-exposure
17 groups in the reinforced-plastics industry were 52 cases of any lymphohematopoietic
18 malignancy (53 expected) (see Table 3-10). In the largest study (the multi-country) the
19 risk of all lymphohematopoietic malignancies increased with average exposure ($P =$
20 0.019) and time since first exposure ($P = 0.012$), but did not increase with increasing
21 cumulative styrene exposure (Kogevinas *et al.* 1994a). No increased risk was observed
22 with duration of employment in the other studies (Kolstad *et al.* 1994, Ruder *et al.* 2004,
23 Wong *et al.* 1994).

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

1 4.1 to 12.5) among workers engaged in synthetic rubber tire manufacture (primarily
2 styrene-butadiene), [but no adjustment for other exposures was attempted].

3 In the cohort of styrene-butadiene rubber workers established by Delzell and colleagues,
4 a slightly increased mortality from all lymphohematopoietic malignancies (SMR = 1.06,
5 95% CI = 0.90 to 1.23, 162 observed deaths) was observed in the 1998 follow-up by
6 (Sathiakumar *et al.* 2005). This cohort included styrene-butadiene rubber workers from a
7 smaller cohort reported by Meinhardt *et al.* and most of the workers from a larger cohort
8 studied by Matanoski and coworkers. There were numerous publications on both cohorts
9 or subpopulations of the cohorts (Matanoski and Delzell), and interpretation of the
10 studies are complicated by overlapping populations, different exposure assessments and
11 different types of analyses.

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

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

14 3.8.5.2 Leukemias

15 *Reinforced plastic workers:* Among the reinforced-plastics workers, Kolstad *et al.* 1994
16 reported a non-significant increased incidence in leukemia (SIR = 1.22, 95% CI = 0.88 to
17 1.65, 42 observed cases) among Danish workers (which overlaps with the international
18 study reported by Kogevinas *et al.* (1994a, 1993). Kogevinas *et al.* reported non-
19 significant increases for myeloid leukemia mortality, but no increase with increasing
20 average or cumulative exposure was observed; a non-significant trend was observed with
21 time since first exposure ($P = 0.094$). In the Danish study, significantly increased
22 mortality from leukemia was observed among workers with more than 10 years after first
23 styrene exposure and for workers with earlier years of first employment (Kolstad *et al.*
24 1994). No relationship between cumulative exposure or duration was observed among the
25 U.S. workers reported by Wong *et al.* (1994). Among the high-styrene-exposure groups
26 in the reinforced-plastics industry, a total of 19 cases of leukemia was observed (19.6
27 expected) (see Table 3-10). In analyses of subtypes of leukemia, the risk of myelogenous
28 leukemia (chronic and acute) was slightly higher than for all leukemia (Kogevinas *et al.*
29 1994a), and increased risk was also seen for myeloid leukemia with chromosomal
30 aberrations in a nested case-control study of the Danish workers (Kolstad *et al.* 1996).

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

5 In the latest follow-up (to 1998) of the most comprehensive cohort of styrene-butadiene
6 workers, the SMR for leukemia among all workers was 1.16 (95% CI = 0.91 to 1.47, 71
7 deaths) (Sathiakumar *et al.* 2005). Compared with workers with other combinations of
8 duration of employment and time since hire, the highest risk of leukemia in this cohort
9 was observed among workers with > 10 years of employment and 20 to 29 years since
10 hire (SMR = 2.58, 95% CI = 1.56 to 4.03, 19 deaths). Among this subgroup, those who
11 had been hired between 1950 and 1959 had the highest risk of leukemia (SMR = 3.92,
12 95% CI = 1.96 to 7.03, 11 deaths (Delzell *et al.* 2006). Statistically significant increased
13 risks of leukemia (SMR ranging from 2.58 to 4.31) were observed among workers
14 involved in production (polymerization and coagulation) job groups) and labor
15 (maintenance and laboratories job groups) (Sathiakumar *et al.* 2005). (Note that
16 production and maintenance workers had high exposure to both styrene and butadiene,
17 and coagulation workers had low to moderate exposure to styrene, but only background
18 exposure to butadiene.) Significant SMRs (approximately 2-fold increased) were also
19 reported among the two highest categories of cumulative levels of styrene exposure.

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

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

16 Statistically nonsignificant increases in relative risk were observed in internal analyses by
17 subtypes of leukemia (CLL, AML, and other) for terciles of styrene exposure; no
18 increasing risks with exposure were observed for CML (Graff *et al.* 2005, Delzell *et al.*
19 2006). (These analyses were restricted to workers over 40 years of age and, in some
20 cases, to > 20 years since hire). In external analyses of CLL, AML, and CML, non-
21 significant increases in CML and CLL were observed, and CLL was significantly
22 increased at cumulative styrene exposures exceeding 61.1 ppm-years (SMR = 3.10, 95%
23 CI = 1.01 to 7.24, 5 deaths).

24 *Other studies:* Among all workers at the four styrene monomer and polymer plants
25 studied, slightly increased mortality was seen for leukemia (10 observed deaths vs. 8.7
26 expected (Bond *et al.* 1992, Frentzel-Beyme *et al.* 1978, Hodgson and Jones 1985,
27 Nicholson *et al.* 1978). A statistically nonsignificant increase in leukemia was observed
28 in the U.S. cohort of styrene monomer and polymer workers (SMR = 1.18, 95% CI =
29 0.54 to 2.24, 9 deaths) (Bond *et al.* 1992). In the study of environmental exposure to

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

3 The 2 case-control studies that addressed leukemia (Flodin *et al.* 1986, Guenel *et al.*
4 2002) had small numbers of exposed subjects (Guenel – 2 cases of leukemia and 9
5 controls, and Flodin – 3 cases of myeloid leukemia and 1 control), [precluding any firm
6 conclusions (despite the high OR found in the study of Flodin *et al.*)].

7 3.8.5.3 Other lymphohematopoietic cancers

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

17 *Styrene-butadiene rubber workers:* In the styrene-butadiene industry, several subtypes of
18 lymphohematopoietic cancers were investigated in the overlapping cohorts established by
19 Matanoski and Delzell *et al.* The nested case-control study from the Matanoski cohort of
20 58 lymphohematopoietic cases and 1,242 controls found two- to three-fold increased
21 risks for lymphoma, lymphosarcoma, and myeloma and styrene exposure (increase of 1
22 ppm in TWA) (Matanoski *et al.* 1997), and the risk of myeloma increased with increasing
23 cumulative exposure to styrene using the step-down regression analysis and taking into
24 account butadiene exposure and other variables. Styrene exposure was not associated
25 with Hodgkin's disease. However, no associations between other types of
26 lymphohematopoietic cancers (lymphosarcoma, Hodgkin's disease, and other lymphatic
27 cancers) were observed in the nested case-control study reported by Santos-Burgoa *et al.*
28 (1992).

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

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

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

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

12 3.8.6 Other sites

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

20 *Prostate:* Ruder *et al.* (2004) reported a significant increase in prostate cancer mortality
21 among reinforced plastic workers; SMRs were elevated in both the high- and low-
22 exposure groups although the SMR was slightly higher in the high-exposure cohort.
23 Mixed results (nonsignificant increases or decreases) were observed in the other cohort
24 studies; however, Gerin *et al.* reported a significant risk (OR = 5.5, 95% CI = 1.4 to 21.8,
25 7 exposed cases and 3 controls) for medium to high styrene exposure in the Canadian
26 case-control study. In the most recent update of the Delzell *et al.* cohort, a slight but non-
27 significant increase in the SMR for prostate cancer was observed (SMR = 1.04, 95% CI =
28 0.88 to 1.21, 154 deaths) (Sathiakumar *et al.* 2005), but no increase in the relative risk of
29 prostate cancer was observed by increasing levels of cumulative styrene exposure in

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

3 *Rectal:* A significantly increased incidence of rectal cancer was observed among the
4 biomonitored workers; the incidence was higher among individuals with > 10-year
5 follow-up, but did not increase with increasing lifetime urinary metabolite levels (Anttila
6 *et al.* 1998). A significant risk (OR = 3.9, 95% CI = 1.2 to 12.9, 5 exposed cases) was
7 also observed in the Canadian case-control study for substantial exposure to styrene
8 (Dumas *et al.* 2000). However, non-significant decreases or null results were reported in
9 most of the other studies. A slight but non-significant increase in the SMR for colorectal
10 cancer was observed by Sathiakumar *et al.* (2005) (SMR = 1.09, 95% CI = 0.94 to 1.25,
11 193 deaths). In an internal analysis of increasing exposure to styrene, the relative risk
12 exceeded 1.0 in 3 of 4 quartiles (1.2, 1.2, 0.6 and 1.5, respectively) but none of the
13 estimates were significant (Delzell *et al.* 2006).

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

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

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

16 [The cohort studies have not attempted to control for confounding factors that affect
17 breast cancer risk (such as body mass index, family history of breast cancer, alcohol use,
18 menopausal status, parity, hormone use, and age at first birth). In addition, cohort studies
19 often do not have sufficient follow-up time to detect effects on incidence or mortality
20 because of the long latency (sometimes in excess of 40 years) from the initiation to
21 detection of breast cancer. A decreased risk of breast cancer was found among women in
22 the cohort study of environmental exposure to styrene, but this study was limited by
23 small numbers of expected (7) and observed (4) deaths from breast cancer, and
24 questionable completeness of follow-up and identification of death certificates (Loughlin
25 *et al.* 1999). Note that a marginally significant increase in the incident risk ratio for breast
26 cancer was observed among a cohort of women army personnel in occupations with
27 medium to high potential exposure to volatile organic compounds (VOC), including
28 potential styrene exposure, (IRR = 1.48, 95% CI = 1.01 to 2.07 [95% CI = 1.03 to 2.12
29 also reported in a table]) compared with women with no or low VOC exposure (Rennix *et*
30 *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 <i>et al.</i>)				United States (Wong <i>et al.</i> 1994)				Denmark (Kolstad <i>et al.</i> 1994, 1995)				Europe ^a (Kogevinas <i>et al.</i> 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.

^aThe 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 <i>et al.</i> , Ruder <i>et al.</i>)				United States ^b (Wong, Wong <i>et al.</i>)				Denmark ^c (Kolstad <i>et al.</i>)				Europe ^d (Kogevinas <i>et al.</i>)				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.

^aWorkers employed in fibrous glass or lamination departments.

^bOpen-mold process workers for more than two years.

^cAll workers employed in companies with 50% to 100% laminators.

^dLaminators, excluding the Danish workers included by Kolstad *et al.*

1 **3.9 Summary**

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

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

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

1 In the styrene-butadiene industry, the cohort studies are among the largest, with the
2 longest follow-up times. The principal methodological challenge is to separate the
3 potentially independent or synergistic effects of butadiene, a known human carcinogen,
4 which is highly correlated with styrene in this industry. Two independent (non-
5 overlapping populations) are available, a small cohort of 6,678 male workers at a rubber
6 tire manufacturing plant (a subset of the workers were engaged in the production of
7 styrene-butadiene and other rubbers) (McMichael *et al.* 1976a) and a larger cohort
8 established by Delzell and colleagues (Delzell *et al.* 1996, 2006) of 13,130 to 16,610
9 styrene-butadiene rubber industry workers from multiple plants in the United States and
10 Canada. The cohort established by Delzell includes most (but not all) of the workers from
11 two cohorts — a 2-plant cohort (Texas) (Meinhardt *et al.* 1982) and an 8-plant cohort
12 originally established by Matanoski and colleagues (United States and Canada) and
13 reported in a series of previous publications (7 of the 8 plants were included in the
14 Delzell cohort). Thus, there is considerable overlap between these populations. Two
15 nested case-control studies (Matanoski *et al.* 1997, Santos-Burgoa *et al.* 1992) of a single
16 group of cases with lymphohematopoietic cancers were available from the Matanoski
17 cohort. The Delzell cohort expanded the previous cohorts to include workers employed
18 from 1943 to January 1, 1991 and followed to 1998, whereas the earlier cohort included
19 workers employed until 1976 and followed until 1982. In addition, the individual study
20 populations were established by different procedures and exclusion criteria (which may
21 partly explain the lack of complete consistency in the number of study subjects across the
22 published studies) and often used different exposure assessments, selection of study
23 subjects, and types of analysis. Two types of analyses were conducted on the Delzell
24 cohort: external analyses reporting on standardized mortality ratios (SMRs) for the total
25 cohort or subsets of the cohorts for multiple cancers sites (Sathiakumar *et al.* 1998,
26 2005), and, secondly, internal analyses of relative risk (RR) estimates for quantitative
27 exposure to styrene and lymphohematopoietic cancers (Delzell *et al.* 2001, 2006,
28 Macaluso *et al.* 2006, Graff *et al.* 2005). (Dimethyldithiocarbamate [DMDTC] was also
29 included as a potential confounder in some analyses of lymphohematopoietic cancer in
30 the Delzell cohort, according to the authors, because of its potential immunosuppressant
31 activity in CD4+ lymphocytes, although its carcinogenicity has not been evaluated

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

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

8 *Lymphohematopoietic cancers*

9 Statistically significant increases were observed for all lymphohematopoietic cancers
10 combined and leukemia among rubber-tire manufacturing workers (McMichael *et al.*
11 1976) and statistically nonsignificant increases were observed for combined
12 lymphohematopoietic cancers and some specific lymphohematopoietic cancers in the
13 Meinhardt and Matanoski cohorts, but the potentially confounding effects of butadiene
14 and other exposures were not analyzed. Two nested case-control studies (using different
15 types of analyses and exposure assessments and the same group of cases) from the
16 Matanoski cohort attempted to evaluate the relative contribution of styrene and butadiene
17 to lymphohematopoietic cancer mortality. Santos-Burgoa *et al.* (1992) found no
18 significant excess risks for combined and specific lymphohematopoietic cancers and
19 mean exposure after controlling for butadiene exposure. Matanoski *et al.* (1997)
20 calculated risks for both average and cumulative exposure to styrene. Taking into account
21 butadiene exposure, and demographic and employment variables in step-down regression
22 analyses, these models found, for an average exposure of 1 ppm vs. no exposure,
23 significant associations for all lymphohematopoietic cancers combined, lymphomas, and
24 myeloma, but not leukemia. For cumulative exposure, significant positive associations
25 between styrene exposure and combined lymphohematopoietic cancers, leukemia, and
26 myeloma were found, with butadiene exposure dropping out of each of the final models
27 except for leukemia.

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

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

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

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

1 employment, but not for workers employed for 1 year or more (Kolstad *et al.* 1994). In
2 the European multi-country cohort (which overlaps with the Danish study), no excess of
3 leukemia mortality was found, and no exposure-response relationships with cumulative
4 or average exposure were observed, although a non-significant trend was observed with
5 time since first exposure (Kogevinas *et al.* 1994a). With respect to other
6 lymphohematopoietic cancers, non-significantly increased risks for non-Hodgkin's
7 lymphoma were found in the Danish and European multi-country cohorts. Positive
8 exposure-response relationships with average styrene exposure and time since first
9 exposure was observed for lymphohematopoietic cancers ($P = 0.019$ and 0.012 ,
10 respectively) and for malignant lymphoma ($P = 0.052$ and 0.072 , respectively) in the
11 European multi-country cohort, but no relationship with cumulative exposure was
12 observed (Kogevinas *et al.* 1994a). No excesses in mortality from any
13 lymphohematopoietic cancers were observed in the two smaller cohort studies (Ruder *et al.*
14 *et al.* 2004 and Wong *et al.* 1994). In the styrene monomer and polymer industries, the risk
15 of lymphohematopoietic malignancies was also increased in most of the studies (as well
16 as the total number of observed cases across studies), but these workers might also have
17 been exposed to benzene.

18 *Pancreatic cancer*

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

1 (10 years after the first measurement) (Anttila *et al.* 1998). However, no increased risk of
2 pancreatic cancer was reported among styrene-butadiene workers (Sathiakumar *et al.*
3 2005).

4 *Esophageal cancer*

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

16 *Other sites*

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

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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, Ponomarev 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).

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

3 4.1.1 Oral

4 Styrene [one impurity (0.3%) was reported in 1 of the 6 batches purchased for the
5 bioassay, but purity was not specified for the remaining 5 batches] was administered by
6 gavage in corn oil to groups of 50 male and 50 female B6C3F₁ mice for 78 weeks (NCI
7 1979a). The mice were approximately 6 weeks old at the beginning of the study. Test
8 groups received styrene at 150 or 300 mg/kg b.w., 5 days per week, while control groups
9 of 20 male and 20 female mice were exposed to corn oil alone. Mice were held for an
10 additional 13 weeks after the last treatment. There was a slight dose-related body weight
11 depression in female mice. Survival in male mice was 78% (high dose), 92% (low dose),
12 and 100% (controls); survival in female mice was 76% (high dose), 80% (low dose), and
13 90% (controls). The Tarone test for dose-related mortality was significant in male mice
14 ($P = 0.003$). Therefore, animals that did not survive at least 52 weeks or died before the
15 first appearance of the tumor(s) of interest were not included in the analysis. The
16 Cochran-Armitage exact trend analysis also indicated a significant dose-response
17 relationship for combined alveolar/bronchiolar neoplasms in male mice. This was
18 supported by an increased incidence of alveolar/bronchiolar neoplasms (adenoma and
19 carcinoma combined) in male mice in the high-dose group compared with controls (Table
20 4-1). Because the incidence of lung tumors in the male vehicle-treated controls (0%) in
21 this study was unusually low compared with historical untreated controls (32 of 271,
22 12%), there was some uncertainty regarding the significance of the lung tumors. NCI
23 (1979a) reported that the historical incidence of these tumors in vehicle control male
24 mice was 0 of 40 (2 studies from Litton Bionetics, including the styrene study); however,
25 this was considered by NCI to be too small a number of animals for meaningful use as
26 historical controls. [The NTP reviewed (for the purpose of this document) lung tumor
27 incidences in historical vehicle controls from NCI studies conducted at other laboratories.
28 However, although the studies were performed at different laboratories, the historical
29 vehicle control animals were from the same source and same study protocol, and the tests
30 were performed in the same chronological window. The selection criteria included data
31 for corn oil vehicle controls for gavage studies in male B6C3F₁ mice conducted prior to

1 1979 with a similar duration (total of 91 weeks) and from the same source as the styrene
2 study. In addition to the two studies from Litton Bionetics (NTP 1979a, 1979b), there
3 were 12 applicable studies conducted by Hazleton Laboratories (NTP 1976a, 1976b,
4 1977, 1978a, 1978b, 1978c, 1978d, 1978e, 1978f, 1978g, 1978h, 1978i). The incidence of
5 combined lung tumors in historical vehicle controls from these 14 studies was 11 of 273
6 (4%). Therefore, the incidence of lung tumors in control male mice in the NCI (1979a)
7 study was not unusually low and support the finding that lung tumors as a result of
8 styrene exposure are statistically significant.] In addition to the lung tumors,
9 hepatocellular carcinomas were reported in male mice but occurred at a higher incidence
10 in controls (20%) than in the treatment groups (6% to 14%). Thus, there were no
11 significant hepatocellular tumor findings in male mice. No other tumors were considered
12 as dose-related. Although there was a significant trend for hepatocellular adenoma ($P =$
13 0.03) in female mice, there were no hepatocellular carcinomas in any female mice, and
14 the NCI did not consider this marginal adenoma effect to be related to styrene. NCI
15 (1979a) concluded that there was suggestive evidence for the carcinogenicity of styrene
16 in male B6C3F₁ mice, but no convincing evidence was obtained for either sex.

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

Table 4-1. Tumor incidences in B6C3F₁ 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]	[<i>P</i> = 0.08]	[<i>P</i> = 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]		[<i>P</i> = 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].

^bIncidences 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].

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

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

- 1 cause for some concern, one adenoma was also observed in a vehicle control male (8.3%)
 2 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 ^c	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 ^c	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.

1 4.1.2 Inhalation

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

12 There was an increased incidence of benign lung tumors (alveolar/bronchiolar adenomas)
13 at several exposure levels in both sexes and malignant lung tumors (alveolar/bronchiolar
14 carcinomas) in high-exposure female mice at the end of the study (Table 4-3). Incidences
15 of adenomas and carcinomas combined were not evaluated by the study authors, but
16 Cohen *et al.* (2002) reported that the combined tumor incidences were significantly
17 higher than controls at exposures of 40, 80, and 160 ppm (male mice) and 20, 40, and 160
18 ppm (female mice). [These results for combined tumor incidences were confirmed by
19 NTP by Fisher's exact test for pairwise comparisons and Cochran-Armitage exact trend
20 test (see Table 4-3).] Cruzan *et al.* (2001) also reported that there was a significant
21 positive trend for benign lung tumors in both sexes and for benign plus malignant tumors
22 in female mice. The incidence of lung tumors was not increased in styrene-exposed mice
23 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]	[<i>P</i> < 0.001]	[NS]	[<i>P</i> < 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]	[<i>P</i> < 0.001]	[<i>P</i> < 0.001]	[<i>P</i> < 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).

^bDue 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.

1 4.1.3 Intraperitoneal injection

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

12 4.2 Rats

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

18 4.2.1 Oral

19 Styrene was administered by gavage in three studies and in the drinking water in one
20 study. These studies are reviewed briefly below and the results are summarized in Table
21 4-5.

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

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

9 NCI (1979a) treated groups of 50 male and female F344 rats with 500, 1,000, or 2,000
10 mg/kg styrene (purity described in Section 4.1.1) in corn oil by gavage 5 days per week
11 for 78 weeks (mid- and high-dose groups). The rats were 6 weeks old at the beginning of
12 the study. Surviving animals were sacrificed after 104 or 105 weeks. Only 6 of 50 male
13 rats in the high-dose group survived past week 53, and only 7 of 50 female rats survived
14 past week 70. Because of poor survival, the high-dose groups were not included in the
15 statistical analysis of tumors. Due to excessive mortality in the high- and medium-dose
16 groups, additional groups of male and female rats were placed on test in week 23. These
17 dosed rats were intubated with styrene at a level of 500 mg/kg for 103 weeks, followed
18 by a 1-week observation period, when no test chemicals were used. Separate vehicle
19 controls were also started for this group. Survival of low- (44/50) and medium-dose rats
20 (47/50) at week 90 was considered adequate by the study authors. No increased tumor
21 incidences were observed in any of the treatment groups.

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

1 Sprague-Dawley rats (7 weeks old at the start of the study) were exposed to styrene
2 (purity \geq 98.9%) in their drinking water for two years (Beliles *et al.* 1985). [This study
3 was identified as the Chemical Manufacturers Association study by Huff (1984) before it
4 was published.] Nominal concentrations were 125 or 250 ppm. The authors noted that the
5 calculated daily doses in this study (7.7 to 14 mg/kg in males and 12 to 20.5 mg/kg in
6 females) were at least an order of magnitude lower than doses used in other chronic oral
7 toxicity studies with styrene, such as the NCI study above (500, 1,000, or 2,000 mg/kg)
8 [solubility of styrene in water limited the dosage]. Chronic toxicity and reproductive
9 performance were evaluated. The test groups included 50 male and 70 female rats, while
10 the control group consisted of 76 males and 106 females. This study also evaluated the
11 effects of styrene on reproductive function through three generations (see Section 5.4.2
12 for reproductive toxicity). The only treatment-related effect identified was a decrease in
13 water consumption. There was no effect on mortality. The authors reported that all
14 tumors observed were either common, spontaneously occurring tumors of Sprague-
15 Dawley rats or were uncommon tumors that affected only individual rats in the treatment
16 groups and concluded that styrene administered in drinking water did not produce
17 deleterious dose-related effects in rats. Tumors were identified only by tissue (number of
18 tissues examined and total number of tumors) [actual tumor rates not reported]. However,
19 Huff *et al.* (1984) reexamined these data and reported specific mammary tumor
20 incidences for fibroadenoma, adenoma, adenocarcinoma, and combined mammary
21 tumors (Table 4-4). The authors reported marginal increase in combined mammary gland
22 tumors (fibroadenoma, adenoma, and adenocarcinoma) in female rats. Incidences were
23 49 of 96 (51%) in controls, 18 of 30 (60%) in the low-dose group and 40 of 60 (66.7%) in
24 the high-dose group. Huff reported that there was a significant dose-related trend ($P =$
25 0.032), and the incidence in the high-dose group was significantly higher than the control
26 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]	[<i>P</i> = 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 <i>et al.</i> 1982, Conti <i>et al.</i> 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 <i>et al.</i> 1985	Sprague-Dawley	<u>Males</u>				No treatment-related increases of any type of tumor reported by study authors. [Limitations: Low doses]
		0 7.7 ^d 14 ^d	76 50 50	104	104	
		<u>Females</u>				
		0 12 ^d 20.5 ^d	106 70 70	104	104	

^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).

1 4.2.2 *Inhalation*

2 Conti *et al.* (1988) exposed groups of 30 male and 30 female Sprague-Dawley rats to
3 styrene vapors (purity 99.8%) in stainless steel inhalation chambers at concentrations of
4 25, 50, 100, 200, or 300 ppm for 4 hours/day, 5 days/week for 52 weeks and held the
5 animals until death. The animals were 13 weeks old at the beginning of the study. The
6 control groups included 60 rats of each sex. There were no significant differences in body
7 weight or mortality between exposed and control groups. A higher incidence, but not
8 statistically significant, of total malignant tumors that was not due to an increase in any
9 specific tumor was observed in male (8 of 30, 26.7%) and female (13 of 30, 43.3%) rats
10 exposed to 100 ppm compared with controls (10 of 60, 16.7% in males and 16 of 60,
11 26.7% in females). Total malignant tumors were not increased at the two highest dose
12 levels. The incidence of malignant mammary tumors was higher in female rats (all
13 exposed groups) compared with controls (Table 4-6). [Therefore, it appears that the
14 reported incidence of malignant mammary tumors was too high, or the incidence of total
15 malignant tumors was too low.] The authors concluded that the increased incidence of
16 malignant mammary tumors in female rats was “treatment-related and statistically
17 significant” and that this study demonstrated a weak carcinogenic effect for styrene.
18 IARC (1994a) considered this study to be inconclusive because of incomplete reporting
19 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]
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]

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.]

1 An unpublished study (Jersey *et al.* 1978) was reviewed by WHO (1983), Huff (1984)
 2 [note that Huff referenced the paper as Dow 1978], ATSDR (1992), McConnell and
 3 Swenberg (1993, 1994), and Cohen *et al.* (2002) and is included here based on
 4 information from these secondary sources. [However, without the original data provided
 5 in the unpublished laboratory report data essential to the interpretation of this study are
 6 missing.] Groups of 96 or 97 male and 96 female Sprague-Dawley rats (7 to 8 weeks of
 7 age) were exposed to 600- or 1,200-ppm styrene (purity 99.5%) for 6 hours/day, 5
 8 days/week. After 2 months, the concentration for the high-dose group was reduced to
 9 1,000 ppm because of excessive toxicity. Interim sacrifices of 5 or 6 animals of each sex
 10 were conducted after 6 and 12 months. Styrene exposure was stopped after 18.3 months
 11 in males and 20.7 months in females because mortality had reached 50%. Animals were

1 observed until their deaths, or 24 months. Survival was lower in males than in females
2 (attributed to a high incidence of chronic murine pneumonia in males). At 24 months, the
3 number of surviving animals was as follows: control group (5 males and 30 females),
4 600-ppm group (18 males and 30 females), 1,000-ppm group (6 males and 22 females)
5 (Cohen *et al.* 2002). Although the incidence of mammary adenocarcinoma in females in
6 the 600-ppm group was 8.2% and was significantly higher than in controls, the authors
7 concluded that there was no causal association with styrene exposure because mammary
8 adenocarcinoma did not occur in the high-dose group, the incidence of mammary
9 adenocarcinoma in the control group (1.2%) was low compared with historical controls
10 (mean of 5.8%), and the range among historical controls (0% to 9%) contained the rate
11 observed in the treatment group. Incidences of mammary fibroadenoma showed no
12 evidence of a styrene effect (WHO 1983). Combined incidences of lymphosarcoma and
13 leukemia in female rats were 1.2% in controls and 7.1% in both exposed groups;
14 incidences in males were 1.2% in controls, 5.8% in the 600-ppm group, and 1.2% in the
15 1,000-ppm group (Table 4-7). Incidences of lymphosarcoma and leukemia were not
16 statistically significant compared with the concurrent controls but were significant when
17 compared with historical controls. [The combined incidence of leukemia and
18 lymphosarcoma in historical controls was not provided; however, these tumors are not
19 typically combined in carcinogenicity studies.] Huff (1984) mentioned that the authors
20 concluded that the data were “suggestive of an association between the exposure of these
21 female rats to styrene vapor and an increased incidence of tumors of the leukemia-
22 lymphosarcoma type. In males, the results are even more inconclusive but tend to support
23 the suggestive association found in the females.” McConnell and Swenberg (1994) noted
24 that “this study was seriously flawed by the presence of chronic murine pneumonia,
25 which caused a high rate of mortality in both control and exposed male rats; it was less a
26 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] ^c			[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] ^c			[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.]

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

1 female rats had decreases in pituitary adenomas and mammary adenocarcinomas
 2 compared with controls (Table 4-8). Incidences of mammary tumors were based on the
 3 total population rather than the number examined because these tumors are rarely found
 4 microscopically when not seen macroscopically (Cruzan *et al.* 1998). There was a
 5 positive trend for testicular tumors, but none of the pairwise comparisons was significant,
 6 and tumor incidences were within the historical control range (0% to 13.5%). Therefore,
 7 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.

^aHistorical 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.]

8 **4.2.3 Parenteral administration**

9 Conti *et al.* (1988) exposed groups of Sprague-Dawley rats to styrene by either i.p. or s.c.
 10 injection. Groups of 40 male and 40 female rats were given four i.p. injections containing
 11 50 mg styrene in olive oil at 2-month intervals. Controls were given i.p. injections of
 12 olive oil. Other groups of 40 male and 40 female rats received a single s.c. injection of 50
 13 mg of styrene in olive oil. Animals were 13 weeks old at the beginning of the study and
 14 were held until death. No treatment-related neoplasms were reported. [The studies were
 15 markedly limited by the low and infrequent doses, short duration of styrene exposure, and
 16 incomplete reporting.]

1 **4.3 Mixtures containing styrene**

2 NCI (1979b) also conducted carcinogenicity studies of a mixture containing 30% β -
3 nitrostyrene and 70% styrene in B6C3F₁ mice and F344 rats. Exposed groups included 50
4 animals of each sex, while control groups included 20 animals of each sex. Mice were
5 administered solutions in corn oil containing 87.5 or 175 mg/kg β -nitrostyrene [204 or
6 408 mg/kg styrene] by gavage 3 days/week for 78 weeks followed by a 14-week
7 observation period. Male rats were administered 150 or 300 mg/kg β -nitrostyrene [350 or
8 700 mg/kg styrene], and female rats were administered 75 or 150 mg/kg β -nitrostyrene
9 [175 or 350 mg/kg styrene] 3 days/week for 79 weeks followed by a 29-week observation
10 period. Control groups were gavaged with corn oil on the same schedule as the treatment
11 groups. All animals were approximately 6 weeks old at the beginning of the study. The
12 authors concluded that a sufficient number of animals survived to the end of the study in
13 all groups. Survival was not significantly affected by exposure in rats (both sexes) or
14 female mice. The probability of survival was dose-related in male mice (90% in controls,
15 86% in the low-dose group, and 66% in the high-dose group). Body weights were
16 depressed in high-dose male rats and female mice. Male mice in the low-dose group had
17 a significantly ($P = 0.016$) increased incidence (11 of 49; 22.4%) of alveolar/bronchiolar
18 adenoma or carcinoma compared with controls (0 of 20) (Table 4-9). The incidence in the
19 high-dose group was 2 of 36 (5.5%) and was not significant by pairwise comparison. No
20 other neoplasms in mice or rats were associated with exposure to the styrene mixture.
21 [However, because of poor survival of the high-dose male mice there were substantially
22 fewer animals at risk for late-occurring lung tumors.] The NCI concluded that “under the
23 conditions of this bioassay, there was no convincing evidence that a mixture of β -
24 nitrostyrene and styrene was carcinogenic in B6C3F₁ mice or F344 rats.”

Table 4-9. Tumor incidences in B6C3F₁ 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).

1 4.4 Styrene metabolites

2 Styrene-7,8-oxide is a primary metabolite of styrene (see Section 1.3) and is listed in the
3 Report on Carcinogens as *reasonably anticipated to be a human carcinogen* based on
4 sufficient evidence in experimental animals (see NTP (2004) for detailed information on
5 the carcinogenicity of styrene oxide). IARC (1994b) also reviewed this compound and
6 concluded that there was “*sufficient evidence* in experimental animals for the
7 carcinogenicity of styrene-7,8-oxide.” Styrene oxide induced high incidences of both
8 benign and malignant tumors of the forestomach in both sexes of rats (three strains
9 tested) and in one strain of mice (IARC 1994b) (see Table 4-10). In addition, Lijinsky
10 (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 <i>et al.</i> 1988, Maltoni <i>et al.</i> 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 Ponomarev <i>et al.</i> 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

1 4.5 Summary

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

1 In mice, gavage studies in both sexes for three strains, an inhalation study in both sexes
2 of one strain, and one i.p. study in females were found in the literature and reviewed.
3 [The oral gavage studies in B6C3F₁ mice (NCI 1979a) and the inhalation studies in CD-1
4 mice (Cruzan *et al.* 2001) were the most informative of the carcinogenicity studies.]

5 Male B6C3F₁ mice had a statistically significant dose-response trend for
6 alveolar/bronchiolar adenoma and carcinoma (combined) that was supported by a
7 significantly increased incidence of these lung tumors in the high-dose group. The
8 authors questioned the significance of these lung tumors because the incidence in the
9 control group was unusually low compared with historical untreated controls. However,
10 the concurrent vehicle controls were within the range of historical vehicle controls from
11 the same source, same study protocol, and same chronological window. Further, the
12 tumor incidence in the high-dose group was significantly increased compared with these
13 historical vehicle controls. A dose-related trend in female B6C3F₁ mice was also
14 observed for hepatocellular adenoma. Significantly increased incidences of
15 alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma
16 (combined) were also observed in male and female CD-1 mice exposed to styrene by
17 inhalation. In each sex, three treatment groups (males, 40, 80, 160 ppm; females, 20, 40,
18 160 ppm) showed increases in these tumors. The high-dose (160-ppm) female mice had
19 an increased incidence of alveolar/bronchiolar carcinoma. A significant trend in
20 hepatocellular adenoma in female mice was also reported, but the pairwise comparison
21 between treated and control animals was not significant.

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

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

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

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

22 Uncertain findings include hepatocellular adenomas in female mice (NCI 1979a) and
23 interstitial testicular tumors in rats (Cruzan *et al.* 1998), both of which were statistically
24 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1978, as cited in Huff 1984, WHO 1983, McConnell and Swenberg 1993, 1994, Cohen <i>et al.</i> 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 <i>et al.</i> 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)

^aThe 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.

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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/cm²/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/cm²/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 ¹⁴C-styrene (4.1 µg/cm²) 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

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

25 5.1.1.2 *Experimental animals*

26 Styrene was absorbed in laboratory rodents exposed to styrene vapors or by oral
27 administration, intraperitoneal injection, and skin application (ATSDR 1992, IARC
28 1994a, 2002). Inhalation studies in rats at concentrations ranging from 50 to 2,000 ppm
29 for 5 hours or 80 to 1,200 ppm for 6 hours indicated rapid uptake with styrene
30 concentrations in blood reaching maximal values at the end of the exposure period. In
31 one study, a 15-fold increase in exposure concentration (80 to 1,200 ppm for 6 hours)

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

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

26 5.1.2 Distribution

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

1 5.1.2.1 Humans

2 The observation that partition coefficients for styrene between body tissues and air are
3 high (4,100 for fat, 84 to 154 for other organs, and 59 for blood) led to the suggestion that
4 styrene would accumulate in subcutaneous fat (IARC 1994a, 2002). However, in a study
5 of styrene-exposed workers, urinary excretion of mandelic acid and phenylglyoxylic acid
6 did not increase during a work week, leading the authors to conclude that styrene did not
7 accumulate (Pekari *et al.* 1993). IARC (2002) noted that pharmacokinetic analysis of the
8 disposition of styrene does not indicate that styrene accumulates in subcutaneous fat.

9 Ramsey *et al.* (1980) exposed four healthy volunteers to 80-ppm styrene for 6 hours and
10 concluded that styrene would not accumulate in the human body. The estimated half-life
11 of styrene in subcutaneous fat in humans is between 2 and 5 days (ATSDR 1992, IARC
12 1994a).

13 5.1.2.2 Experimental animals

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

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

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

16 5.1.3 *Metabolism*

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

22 5.1.3.1 *Humans*

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

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

8 Korn *et al.* (1994) reported a linear correlation between the concentrations of styrene-7,8-
9 oxide in the blood of workers and the concentration of styrene in air. The steady-state
10 level of styrene-7,8-oxide was about 1 µg/L at styrene concentrations of 20 ppm.
11 Johanson *et al.* (2000) exposed 4 healthy male volunteers to 50-ppm styrene for 2 hours
12 during light physical activity. Maximum concentrations of styrene-7,8-oxide in blood
13 ranged from 2.5 to 12.2 nM and were observed in the first samples collected shortly after
14 exposure had stopped. No styrene-7,8-oxide was detected in blood samples collected 23.5
15 hours after exposure. Minor styrene metabolites identified in humans include mercapturic
16 acid derivatives of styrene-7,8-oxide (Maestri *et al.* 1997) (which arise from glutathione
17 conjugation of styrene-7,8-oxide), 4-vinylphenol (4-hydroxystyrene) (Pfäffli *et al.* 1981),
18 1-phenylethanol (Korn *et al.* 1985), 2-phenylethanol (Korn *et al.* 1985), and glucuronic
19 acid and sulfur conjugates of hydroxylated styrene metabolites (Manini *et al.* 2002b).
20 Formation of 4-vinylphenol indirectly indicates intermediate formation of the 3,4-arene
21 oxide; formation of 2-vinylphenol (not shown in Figure 5-1), indirectly indicates
22 intermediate formation of styrene-2,3-oxide. Urinary 4-vinylphenol sulfates and
23 glucuronates have been identified in volunteers and occupationally exposed workers, and
24 this metabolic pathway was shown to account for approximately 0.5% to 1% of the total
25 excretion of styrene metabolites (Manini *et al.* 2003).

26 5.1.3.2 *Experimental animals*

27 Metabolism of styrene in various animal species has been reviewed by IARC (1994a,
28 2002) and by Sumner and Fennel (1994). As in humans, the first step in metabolism is
29 usually the epoxidation of styrene to styrene-7,8-oxide in a NADPH-dependent reaction
30 catalyzed by CYP enzymes (Figure 5-1). Styrene-7,8-oxide is further metabolized to

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

9 A second metabolic pathway results in formation of 4-vinylphenol, which is produced in
10 very small amounts in rats (0.1% of styrene dose) (Bakke and Scheline 1970) and
11 humans (Manini *et al.* 2003); this pathway is postulated to involve styrene-3,4-oxide as
12 an intermediate (Pantarotto *et al.* 1978). No 4-vinylphenol was detected in *in vitro*
13 experiments with mouse and rat lung and liver microsomal preparations incubated with
14 styrene, but the authors suggested that rapid metabolism of 4-vinylphenol might explain
15 their failure to detect the metabolite (Carlson *et al.* 2001). When the metabolism of
16 4-vinylphenol was tested in the same system, the metabolic rate was 3 times as high in
17 mouse liver microsomes as in rat liver microsomes and 8 times as high in mouse lung
18 microsomes as in rat lung microsomes. Boogaard *et al.* (2000a) reported that the
19 percentage of ¹⁴CO₂ derived from ring-labeled styrene was 3 to 4 times as high in mice as
20 in rats and suggested that this might indicate formation of reactive ring-opened
21 metabolites in mouse lung, which would likely involve ring oxidation, as postulated for
22 the formation of 4-vinylphenol. Differences in styrene metabolites formed by ring
23 oxidation have been proposed as a possible explanation for the interspecies differences in
24 susceptibility to lung tumors in experimental animals (see Sections 5.1.3.5, 5.2.2.2, and
25 5.5.3).

26 5.1.3.3 Tissue type, lung cell types, and metabolism

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

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

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

^aAll values are mean ± SE.

^bCalculated on the basis of total number of nucleated cells.

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

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

4 Human lung also contains Clara cells (primarily in the bronchiolar epithelium) but the
5 morphology is different from that seen in rodents. Pinkerton *et al.* (1997) reported that
6 the most striking difference was the low proportion of agranular endoplasmic reticulum
7 in human nonciliated bronchiolar epithelial cells (3.1% of the cellular components)
8 compared with 55% in the mouse and 66% in the rat. Human terminal airways do not
9 have significant numbers of Clara cells; however, the contribution of Clara cells to the
10 proliferation compartment of normal human tracheobronchial epithelium is substantial,
11 demonstrating a role of the Clara cell in the maintenance of the normal epithelium of the
12 distal conducting airways in humans. This concept was demonstrated in the study by
13 Boers *et al.* (1999). These authors evaluated the number of Clara cells from normal tissue
14 taken from seven lungs obtained by autopsy. The number of Clara cells in the terminal
15 and respiratory bronchioles were $11 \pm 3\%$ and $22 \pm 5\%$, respectively. The proximal
16 airway epithelium (bronchi and bronchioles) was virtually devoid of Clara cells. The
17 overall proliferation compartment of the conducting airway epithelium was $0.83 \pm$
18 0.47% ; the contribution of Clara cells was 9%. In the terminal bronchioles 15% of
19 proliferating airway epithelial cells were Clara cells, and in the respiratory bronchioles
20 this percentage increased to 44%.

21 5.1.3.4 *Metabolic enzyme activity in human lung*

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

1 Human lung has been reported to contain either mRNA or protein for the following P450
2 isozymes: CYP1A1, CYP1A2, CYP2A6, CYP2A13, CYP1B1, CYP2B6, CYP2C8,
3 CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2F1, CYP2J2, CYP2S1, CYP3A4,
4 CYP3A5, CYP4B1, CYP5A1, CYP7B1, CYP8A1, CYP27, and CYP51 (Ding and
5 Kaminsky 2003, Hukkanen *et al.* 2002, Karlgren *et al.* 2005, Nishimura *et al.* 2003,
6 Pelkonen and Raunio 1997, Seliskar and Rozman 2007, Somers *et al.* 2007, Zhang *et al.*
7 2006). Although levels of most P450 enzymes are reported to be lower in lung compared
8 with liver (Somers *et al.* 2007), CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1
9 are preferentially expressed in the lung (Ding and Kaminsky 2003, Thum *et al.* 2006).

10 Xenobiotic metabolism in human lung occurs primarily in bronchial epithelial cells, Clara
11 cells, type II pneumocytes, and alveolar macrophages; while in rodents and rabbits,
12 metabolism is highest in Clara cells and type II pneumocytes. The CYP2F1 isoform of
13 cytochrome P450 is the human homolog of the Cyp2f2 isoform expressed in mouse lung
14 (see below) and the CYP2F4 isoform expressed in rat lung (Baldwin *et al.* 2005). The
15 presence of a cDNA for CYP2F1 in a human lung library was first reported by Nhamburo
16 *et al.* (1990). The mRNA for CYP2F1 has been shown by RT-PCR amplification to be
17 present in human lung tissue and broncho-alveolar macrophages (Raunio *et al.* 1999) and
18 in human bronchial biopsy and trachea and lung tissue (Bieche *et al.* 2007, Thum *et al.*
19 2006).

20 Sheets *et al.* (2004) reported that A549 human alveolar epithelial type II
21 (adenocarcinoma) lung cells were capable of metabolizing benzene, and the activity
22 decreased significantly (51%; $P < 0.05$) in the presence of 5-phenyl-1-pentyne (5P1P), a
23 P450 inhibitor. 5-Phenyl-1-pentyne is an effective inactivator of CYP2E1 as well as
24 CYP2F2 and CYP2F1 (Roberts *et al.* 1998, Simmonds *et al.* 2004). The authors
25 concluded that CYP2F1 was important in benzene metabolism in this human lung cell
26 line. BEAS-2B cells overexpressing CYP2F1 also were reported to have a significant (P
27 < 0.05) increase in cytotoxicity resulting from bioactivation of 3-methylindole to 3-
28 methyleneindolenine (Nichols *et al.* 2003).

1 5.1.3.5 Metabolic enzyme studies

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

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

22 Nakajima *et al.* (1994a) compared the rate of formation of styrene glycol from styrene in
23 human, rat, and mouse liver microsomes, and in human lung microsomes. At low styrene
24 concentrations (0.085 mM), the rate was highest in mouse liver microsomes (2.43 ± 0.29
25 nmol/(mg of protein·min)) and lowest in humans (0.73 ± 0.45 nmol/(mg of protein·min));
26 however, at a higher styrene concentrations (1.85 mM), the rate was highest in the rat
27 (4.21 ± 0.72 nmol/(mg of protein·min)) but remained lowest in humans (1.91 ± 0.84
28 nmol/(mg of protein·min)). The rate of styrene glycol formation in human lung was less
29 than 1% of that in human liver. The specific P450 forms responsible for the metabolic
30 activity were determined by analyzing cDNA-expressed individual P450 forms produced

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

15 Fukami *et al.* (2008) reported that CYP2A13, a human cytochrome P450 expressed
16 predominantly in the respiratory tract, had the highest catalytic activity for the formation
17 of styrene-7,8-oxide from styrene when compared with CYP2A6 and CYP2E1. These
18 enzymes have overlapping substrate selectivity. The CL_{int} values calculated from the
19 initial slope of velocity plotted against the substrate concentration values were 46.8 for
20 2A13, 17.2 for 2A6, and 18.5 for 2E1.

21 The effects of CYP-specific inhibitors on formation of *R*- and *S*-enantiomers of styrene-
22 7,8-oxide were investigated by Hynes *et al.* (1999) through the use of enriched Clara cell
23 and type II alveolar cell fractions from lungs of male CD-1 mice and male Sprague-
24 Dawley rats. Cyp2e1 and Cyp2f2 were found to be the most important isoforms. Hynes *et al.*
25 reported that the mouse Clara cell preparation metabolized styrene to both
26 enantiomers. CYP-specific inhibitors (diethyldithiocarbamate for Cyp2e1, 5-phenyl-1-
27 pentyne for Cyp2f2, α -naphthoflavone for Cyp1a, and α -
28 methylbenzylaminobenzotriazole for Cyp2b) were used to identify the cytochromes
29 responsible. The Cyp1a inhibitor did not inhibit styrene-7,8-oxide formation, and the
30 Cyp2b inhibitor had only a minor effect. 5-Phenyl-1-pentyne inhibited formation of both

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

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

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

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

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

5 Carlson (2004a) reported that wild-type mice were more susceptible to styrene-induced
6 hepatotoxicity than *Cyp2e1*-knockout mice, but there was no significant difference in the
7 response when styrene-7,8-oxide was administered. These results suggest that *Cyp2e1* is
8 important for bioactivation of styrene in the liver. However, Carlson (2003) reported that
9 there was little or no difference in the rate of metabolism of styrene to styrene-7,8-oxide
10 by hepatic microsomes from wild-type and knockout mice. Sumner *et al.* (2001)
11 compared styrene metabolism in *Cyp2e1*-knockout and wild-type mice *in vivo* and
12 reported that the knockout mice excreted more total urinary metabolites than the wild-
13 type mice. Carlson (2003) rejected the conclusion that these data indicated that *Cyp2e1*
14 was not important in styrene metabolism because this would contradict findings from
15 many previous studies. Carlson concluded that the data more likely indicate that other
16 enzymes must be contributing to styrene metabolism in knockout mice. Carlson (2004a)
17 also noted the reason for this disconnect was unclear, but thought it might be related to
18 kinetic factors associated with styrene metabolism within the liver of the intact animal.

19 Carlson (2003), (see Table 2 in Carlson 2003) reported that styrene metabolism by
20 pulmonary microsomes in *Cyp2e1*-knockout mice is about one-half that in wild-type
21 mice. *Cyp2f2* was also important for metabolism in mouse lung based on inhibition of
22 styrene metabolism by the *Cyp2f2* inhibitor 5-phenyl-1-pentyne. The same inhibitor
23 inhibited the pulmonary cytochrome P450 metabolism of styrene in mice *in vivo* and
24 prevented an increase in cell replication rates (Green *et al.* 2001a). *Cyp1a* and *Cyp2b*
25 were considered to play only minor roles in styrene metabolism because of the small
26 inhibitory effect with α -naphthoflavone, an inhibitor of *Cyp1a*, and α -
27 methylbenzylaminobenzotriazole, an inhibitor of *Cyp1b* (Carlson *et al.* 1998). Nakajima
28 *et al.* (1994b) reported that metabolism by rat lung microsomes was inhibited only by
29 anti-CYP2B1/2 (which probably corresponds to CYP2B1). In *Cyp2e1*-knockout studies,
30 pulmonary toxicity of styrene was similar in both wild-type and knockout mice, which

1 supports previous studies that indicated the importance of styrene metabolism by other
2 enzymes such as Cyp2f2 in the lung (Carlson 2004a).

3 Carlson *et al.* (2001) did not detect 4-vinylphenol when styrene was incubated with
4 hepatic and pulmonary preparations from rats or mice. However, these tissue preparations
5 were shown to have considerable 4-vinylphenol metabolizing ability when incubated with
6 4-vinylphenol in the presence of NADPH. The rate of metabolic activity in mouse liver
7 microsomes was 3 times faster than in rat liver microsomes, and the rate in mouse lung
8 microsomes was 8 times faster than in rat lung microsomes. Treatment with pyridine, an
9 inducer of Cyp2e1, caused a significant increase in 4-vinylphenol metabolism in the liver
10 but not the lung. Furthermore, 4-vinylphenol metabolism was significantly decreased in
11 mouse liver and lung microsomes treated with diethyldithiocarbamate, an inhibitor of
12 Cyp2e1, or 5-phenyl-1-pentyne, an inhibitor of Cyp2f2. This study also indicated that
13 glutathione conjugation was involved in 4-vinylphenol metabolism, with the highest
14 activity in mouse lung, with or without the addition of NADPH. Carlson (2002) also
15 showed that when rats and mice were pretreated with diethyldithiocarbamate or 5-phenyl-
16 1-pentyne, the hepatotoxicity and pneumotoxicity of 4-vinylphenol were prevented or
17 greatly decreased. These data suggest that the toxicity of 4-vinylphenol in the liver and
18 lungs was due to a metabolite rather than to the parent compound. Vogie *et al.* (2004)
19 examined the microsomal metabolism of 4-vinylphenol in wildtype and *Cyp2e1*-
20 knockout mice and reported no marked differences in the rates of microsomal metabolism
21 prepared from the lung and liver of mice with either genotype. The knockout mice were
22 more susceptible to hepatotoxicity than wild-type mice but there was no significant
23 difference in pneumotoxicity. Thus, in contrast to the findings of Carlson, the animals
24 that were unable to metabolize 4-vinylphenol through the Cyp2e1 pathway were more
25 susceptible. Vogie *et al.* stated that the reason for the discrepancy was unknown, but
26 could be related to inhibition of other cytochrome P450 enzymes by
27 diethyldithiocarbamate, or it could have protected against hepatotoxicity by a mechanism
28 not related to Cyp2e1. The rate of metabolism of 4-vinylphenol metabolism was the same
29 in wild-type and *Cyp2e1*-knockout mice indicating that cytochromes P450 other than
30 Cyp2e1 play an important role (Carlson 2004b). This study also showed that the greatest

1 inhibition of enzymatic activity occurred with diethyldithiocarbamate, even in knockout
2 mice, and suggests that it must inhibit other P450 cytochromes in addition to Cyp2e1.

3 5.1.3.6 *Detoxification of styrene metabolites*

4 Only a very small fraction of the styrene-7,8-oxide formed from styrene in the liver
5 reaches the systemic circulation. The vast majority is immediately hydrolyzed
6 enzymatically, as shown by epidemiologic studies (Korn *et al.* 1994) and based on
7 theoretical considerations (Arand *et al.* 1999, Oesch *et al.* 2000). The hydrolysis of
8 styrene-7,8-oxide is efficiently carried out by microsomal epoxide hydrolase (mEH).
9 Although the enzyme has a relatively low turnover, it is able to inactivate genotoxic
10 substrates very rapidly via formation of a covalent intermediate, an enzyme-substrate
11 ester, which is subsequently hydrolyzed in a slow, rate-limiting step (Arand *et al.* 1999,
12 Tzeng *et al.* 1998). Overall clearance of styrene-7,8-oxide is efficiently accomplished by
13 liver mEH; however, detoxification outside the liver is usually less efficient, because it
14 depends on local mEH levels. Oesch *et al.* (2000) predicted that the local styrene-7,8-
15 oxide steady-state level will rise sharply as soon as the rate of styrene-7,8-oxide
16 formation exceeds the local capacity for enzymatic hydrolysis of styrene-7,8-oxide. As a
17 result, tissues that produce the relevant CYP and activate styrene to styrene-7,8-oxide in
18 sufficient amounts, but have low mEH activity, may be more susceptible to styrene-
19 mediated genotoxicity than would be predicted from the systemic styrene-7,8-oxide load
20 deduced from the biomarkers measured in blood. The lung, which is the primary entry
21 site for styrene into the human body, contains cell types that produce styrene-activating
22 CYP isoenzymes (e.g., Clara cells), but it has significantly lower styrene-activating
23 activity and mEH activity than the liver.

24 The second pathway for styrene-7,8-oxide detoxification, the formation of glutathione
25 conjugates and PHEMAs (which constitute less than 1% of styrene metabolites in
26 humans) was reported first in rodents (Delbressine *et al.* 1981) and later in humans
27 (Maestri *et al.* 1997). Although GSH conjugation is a minor detoxification route (Maestri
28 *et al.* 1997), it may become important in extrahepatic tissues with low mEH activity, such
29 as lungs and blood-forming organs, as high levels of GST are found in erythrocytes
30 (Henderson and Speit 2005). Several studies have suggested that polymorphisms in

1 GSTM1 may influence metabolite excretion; higher levels of mandelic acid and
2 phenylglyoxylic acid urinary metabolites (Teixeira *et al.* 2004) or lower levels of
3 phenylethyl mercapturic acids (De Palma *et al.* 2001, Haufroid *et al.* 2002b) were found
4 in styrene-exposed *GSTM1*-null individuals than in wild-type individuals. Studies on
5 *GSTT1* polymorphisms are conflicting (Norppa 2003).

6 5.1.4 Excretion

7 The primary route of styrene excretion in both humans and laboratory rodents is the urine
8 (IARC 1994a); however, the metabolic profiles differ among species (as discussed
9 above). Almost all of the absorbed styrene is excreted as urinary metabolites; however, a
10 small fraction (< 5%) may be eliminated as unchanged styrene in exhaled air or urine
11 (ATSDR 1992, IARC 2002). Mandelic acid and phenylglyoxylic acid (see Table 1-3) are
12 the primary urinary metabolites, accounting for as much as 95% to 98% of the total
13 (Manini *et al.* 2002b). Glutathione conjugates generally account for 1% or less of the
14 absorbed dose (IARC 1994a).

15 5.1.4.1 Humans

16 Elimination of styrene from blood was biphasic in human volunteers, indicating a two-
17 compartment pharmacokinetic process; the half-lives were 0.58 hours for the rapid phase
18 and 13.0 hours for the slow phase (Ramsey *et al.* 1980). Urinary elimination of mandelic
19 acid and phenylglyoxylic acid also were reported to be biphasic in styrene-exposed
20 workers (IARC 1994a); the half-lives for both were 2.5 hours for the rapid phase and
21 30 hours for the slow phase (Wieczorek and Piotrowski 1988). Guillemin and Berode
22 (1988) reviewed data on clearance of these metabolites and also reported that clearances
23 were biphasic. Half-lives for mandelic acid ranged from 3.9 to 9.4 hours during the first
24 20 hours post-exposure and from 16.6 to 26.5 hours after 20 hours post-exposure. Half-
25 lives for phenylglyoxylic acid averaged 10.5 ± 1.4 hours during the first 50 hours post-
26 exposure in one study reviewed by Guilleman and Berode and ranged from 21.5 to 26.7
27 during the period from 20 to 200 hours post-exposure in a second study.

28 IARC (1994a) reported that 0.7% to 4.4% of inhaled styrene was eliminated unchanged
29 in exhaled air. Unchanged styrene also was reported to be excreted in urine by styrene-

1 exposed workers, but the concentration in urine was only about one-tenth that in blood
2 (Guillemin and Berode 1988, IARC 1994a).

3 Pfaffli *et al.* (1981) reported that they could detect 4-vinylphenol in the urine of styrene-
4 exposed workers but not in nonexposed individuals by GC/MS, but the level detected was
5 only 0.3% of the level of mandelic acid in the same individuals. Johanson *et al.* (2000)
6 exposed four healthy male volunteers to 50-ppm styrene for 2 hours. Based on the
7 relationship of mandelic acid and 4-vinylphenol reported by Pfaffli *et al.* (1981),
8 Johanson *et al.* estimated that the maximum level of 4-vinylphenol in their subjects
9 would be about 0.004 mM [below the detection limit]. Manini *et al.* (2003) used liquid
10 chromatography electrospray tandem mass spectrometry to measure 4-vinylphenol
11 conjugates in urine of workers exposed to styrene and in volunteers exposed to 50 mg/m³
12 styrene. Urinary 4-vinylphenol conjugates (glucuronates and sulfates) represented about
13 0.5% to 1% of the total excretion of styrene metabolites and were significantly correlated
14 with airborne styrene ($r = 0.607$, $P < 0.001$) and the sum of mandelic acid and
15 phenylglyoxylic acid ($r = 0.903$; $P < 0.001$) in end-of-shift samples for workers.

16 5.1.4.2 *Experimental animals*

17 In rats, elimination of styrene from blood was reported to be biphasic over a period of 6
18 hours (ATSDR 1992, IARC 1994a). Sumner *et al.* (1997) reported that after inhalation
19 exposure to styrene at 250 ppm for 1 to 5 days, male F344 rats, male CD-1 mice, and
20 male B6C3F₁ mice eliminated most of the absorbed styrene in the urine. Following a
21 single 6-hour exposure, elimination was faster in rats (89% within 12 hours) and CD-1
22 mice (83% within 12 hours) than in B6C3F₁ mice (59% within 12 hours). The slower
23 elimination in B6C3F₁ mice was considered to be consistent with the higher liver toxicity
24 in these mice. However, when the animals were exposed for 3 to 5 days, elimination in
25 all three groups was about 88% within 12 hours. The increased excretion in B6C3F₁ mice
26 with longer-term exposure was consistent with induction of styrene metabolism and with
27 the absence of ongoing acute necrosis following multiple exposures. When CD-1 mice
28 and male Sprague-Dawley rats were exposed to ¹⁴C-labeled styrene by nose-only
29 inhalation, the primary route of excretion was in the urine ($75 \pm 7\%$ of inhaled styrene
30 retained by rats and $63 \pm 9\%$ of that retained by mice), and only a small fraction was

1 eliminated in the feces of either species (Boogaard *et al.* 2000a). The species differed in
2 exhalation of $^{14}\text{CO}_2$, which in two separate experiments accounted for approximately 2%
3 of retained styrene in rats and 6.4% and 8% in mice. Mice also had higher nonspecific
4 binding of radiolabeled styrene in nasal passages and lung than rats.

5 **5.2 Toxicity**

6 The toxicity of styrene has been reviewed (ATSDR 1992, Bond 1989, IARC 1994a,
7 2002). The acute toxicity of styrene in laboratory animals and in humans is considered
8 low to moderate. The oral LD_{50} for styrene in rats is 5,000 mg/kg and the inhalation LC_{50}
9 is 2,770 ppm (2-hour exposure). The LD_{50} in mice is 320 mg/kg for oral exposure, 660
10 mg/kg for i.p. injection, and 90 mg/kg for i.v. injection. The inhalation LC_{50} in mice is
11 4,940 ppm (4-hour exposure). The major acute toxic effects of styrene include irritation
12 of the skin and respiratory tract and effects on the central nervous system (CNS).

13 **5.2.1 Humans**

14 Drowsiness, listlessness, muscular weakness, and unsteadiness are common signs of
15 systemic styrene intoxication in humans (Bond 1989). Skin, eye, throat, and respiratory
16 tract irritation have been reported in styrene-exposed workers (IARC 2002). Direct skin
17 contact with liquid styrene has caused erythema, dermatitis, and blistering. Minamoto *et*
18 *al.* (2002) conducted patch tests on 29 fiberglass-reinforced-plastics workers. Of the 22
19 workers who reported skin problems, one had a positive patch test to styrene. In a study
20 where human volunteers were exposed to styrene concentrations of 51 to 376 ppm for 1
21 to 7 hours, signs of styrene toxicity (including eye and nasal irritation, nausea, and
22 headaches) occurred only in subjects exposed to 376 ppm (Bond 1989). In another study
23 reviewed by Bond, subjects exposed to 800 ppm experienced immediate irritation of the
24 nose and throat and increased nasal secretions. Respiratory tract irritation was reported in
25 humans exposed for short durations and airflow restriction in those exposed for long
26 durations; however, the concentrations and durations were not fully defined. Röder-
27 Stolinski *et al.* (2008) investigated the mechanisms responsible for styrene-induced
28 inflammatory effects using a human alveolar epithelial cell line (A549). Styrene
29 stimulated the expression of inflammatory mediators, including the chemotactic cytokine
30 monocyte chemoattractant protein-1 (MCP-1) in these cells. MCP-1 expression and

1 glutathione S-transferase [a marker of oxidative stress] was associated with a
2 concentration dependent pattern of NF- κ B activation. NF- κ B is a pivotal intracellular
3 signaling pathway involved in inflammatory responses. Treatment with an NF- κ B
4 inhibitor and an antioxidant were effective in suppressing styrene-induced MCP-1
5 secretion.

6 Respiratory effects from occupational exposure to styrene include bronchitis, asthma, and
7 pneumonia. Chronic bronchitis has been reported in workers exposed to styrene
8 concentrations greater than 100 mg/m³ [23 ppm], and increased mortality from
9 pneumonia was associated with styrene exposure among 40,000 men and women
10 employed in 660 European reinforced-plastics manufacturing factories; however, no
11 increased mortality from bronchitis, emphysema, or asthma occurred (IARC 2002). In a
12 more recent study (reviewed by IARC 2002) of workers in the reinforced-plastics and
13 composites industry in the United States, there was no relationship between exposure to
14 styrene and mortality from non-malignant respiratory disease.

15 Effects of styrene exposure on the nervous system, either central or peripheral, have been
16 reported mostly for concentrations of 100 ppm or above (IARC 2002). The effects
17 included decreased nerve conduction velocities and electroencephalographic,
18 dopaminergic, functional, and psychiatric anomalies. At concentrations below 100 ppm,
19 reports of effects have been mixed, with some researchers finding no effects and others
20 reporting memory and neurobehavioral disturbances at concentrations in the range of 10
21 to 30 ppm. In addition to the effects of styrene on reaction time, color vision, and hearing,
22 researchers also have studied the possible effects of styrene exposure on taste. However,
23 Dalton *et al.* (2003) did not find any evidence for an impairment of olfactory function in
24 a group of fiberglass-reinforced-plastics workers.

25 Benignus *et al.* (2005) conducted a meta-analysis of the relationship of long-term
26 exposure to styrene and two measures of CNS function: reaction time and color vision.
27 There was a statistically significant relationship between cumulative exposure to styrene
28 and an increased choice reaction time as well as an increased color confusion index.
29 These authors estimated that 8 work-years of exposure to 20-ppm styrene (the ACGIH

1 limit) produces a 6.5% increase in choice reaction time and an increase in the color
2 confusion index equivalent to 1.7 additional years of age in men (the color confusion
3 index in men increases with age at the rate of about 10% of baseline every 13 years).

4 Color vision was reported to be impaired in several studies reviewed by IARC (2002),
5 and it was proposed that this effect reflects changes in neural functioning along optic
6 pathways. Effects were seen at concentrations of styrene as low as 20 ppm; however, one
7 study reported that the effects of styrene on color vision were reversed after 4 weeks in a
8 styrene-free environment. In a study of 108 workers in Swedish reinforced-plastics
9 plants, Iregren *et al.* (2005a) concluded that there was a “strong indication” that color
10 vision was negatively affected in workers with exposure below the Swedish occupational
11 exposure limit of 90 mg/m³ [21 ppm]. Confounding effects of age and higher past
12 exposure levels were also considered by the authors, but they did not consider these
13 factors sufficient to explain all of the differences observed.

14 Significant changes in hearing thresholds at high frequencies have been reported in
15 workers exposed to styrene in some studies (IARC 2002). Although several studies
16 reviewed by IARC did not find effects on hearing threshold at styrene concentrations
17 below 150 mg/m³ [35 ppm], one study reported disturbances in the central auditory
18 pathways in 7 of 18 workers exposed for 6 to 15 years to styrene at concentrations below
19 110 mg/m³ [25 ppm]. Several recent publications have reviewed the relationship between
20 styrene exposure and hearing loss. Hoet and Lison (2008) reported that styrene appears to
21 be ototoxic in rats, but the human data were insufficient to support a clear conclusion.
22 Sliwinska-Kowalska *et al.* (2007) reported the findings of a scientific workshop that
23 reviewed the ototoxic effects of organic solvents. Seven of nine occupational studies of
24 styrene-only exposure (primarily in the glass fiber–reinforced-plastics industry) showed
25 evidence of hearing loss. Measurements varied among the studies but included pure tone
26 audiometry, high-frequency hearing loss, and central hearing tests. Although one of the
27 primary conclusions from the workshop was that styrene is a risk factor for hearing loss,
28 the authors concluded that the data were not sufficient to derive a dose-response
29 relationship. Johnson (2007) also reviewed these nine studies and noted that the reported
30 effects occurred at concentrations below the current TLV values (20 to 50 ppm) but the

1 authors considered the effects to be negligible. Fuente and McPherson (2006) also
2 reviewed the literature on solvent exposure and hearing loss. They reported that a positive
3 linear relationship was seen between an average working-life exposure to styrene and
4 hearing thresholds at 6,000 and 8,000 Hz. These authors also noted that there was an
5 additive effect on hearing thresholds with exposure to both styrene and noise. Johnson *et*
6 *al.* (2006) reported audiological findings in 313 workers from fiberglass and metal-
7 product manufacturing plants. Workers exposed to noise and styrene had significantly
8 poorer pure-tone thresholds in the high-frequency range than controls or noise-only
9 exposed workers.

10 Toppila *et al.* (2006) noted that styrene is both ototoxic and neurotoxic; thus, styrene
11 exposure could affect postural stability. These authors investigated the effects of low
12 concentrations of styrene on postural stability among 252 male Finnish fiberglass-
13 reinforced plastic boat manufacturers. Smoking history, postural stability, and urine
14 mandelic and phenylglyoxylic acid concentrations were determined. Breathing zone
15 measurements of styrene were measured for 148 workers. Mean styrene concentrations
16 for the age-matched workers were 21 mg/m³ [4.8 ppm] for nonlaminators and 108 mg/m³
17 [25 ppm] for laminators. Their analysis included 88 matched pairs and indicated that
18 postural stability among boat laminators was impaired compared with nonlaminators. The
19 impairment occurred among young workers and worsened with age.

20 Hepatic and renal effects of styrene exposure were mixed or absent in older studies, but
21 more recent reports have found alterations in hepatic clearance of bilirubin and in hepatic
22 alanine and aspartate transaminase activities (IARC 2002). Urinary markers for renal
23 toxicity are reported to be only weakly correlated with styrene exposure.

24 The early studies that examined the effects of styrene exposure on the hematopoietic and
25 immune systems failed to find consistent functional changes (IARC 2002). One study
26 found no differences in hemoglobin, erythrocyte, and leukocyte concentrations in 84
27 workers exposed to styrene concentrations of 50 to 500 ppm for 1 to 36 years. Another
28 study found no evidence of hematological abnormalities in 163 workers in a styrene-
29 butadiene synthetic rubber manufacturing plant. More recent studies reviewed by IARC

1 reported a 30% increase in the number of peripheral blood monocytes in workers exposed
2 to 13-ppm styrene, and an exposure-related decrease in both the mean corpuscular
3 hemoglobin and neutrophil concentrations among 221 workers in the reinforced-plastics
4 industry that were exposed to 1 to 100 ppm for 1 to 20 years. Other studies reported
5 effects on the immune system, including a reduction in total T lymphocytes and T-helper
6 lymphocytes along with an increase in natural killer cells, and alterations in the cell-
7 mediated immune response of T lymphocytes. Changes in lymphocyte subpopulations
8 were observed mainly at concentrations greater than 50 ppm. Biro *et al.* (2002)
9 investigated the immunotoxicity of styrene in 10 styrene-exposed workers compared with
10 29 healthy controls. The data indicated that changes in the expression of surface antigens
11 on peripheral lymphocytes were correlated with exposure. The styrene-exposed group
12 had a significant decrease in the level of CD25+ CD4+ lymphocytes (activated helper T
13 cells) with a concomitant increase in the level of CD45RO+ CD4+ lymphocytes (memory
14 helper T cells), suggesting a shift from activated to memory helper T cells. The styrene-
15 exposed group also had a slightly higher ratio of CD4+ lymphocytes to CD8+ T
16 lymphocytes, which the authors concluded was caused by smoking as this was seen
17 among all smokers compared with nonsmokers, and the styrene-exposed group had a
18 higher percentage of smokers than the control group,

19 Plotnick and Weigel (1979) suggested that a relationship may exist between the
20 distribution of styrene and/or its metabolites in the pancreas and the increased glucose
21 tolerance reported in workers. Chmielewski (1976) reported a statistically significant
22 increased glucose tolerance in workers exposed to styrene for 1 year; however, the
23 increase was not significant in workers exposed for 10 years. Some epidemiological
24 studies have reported pancreatic cancer in workers exposed to styrene (see Section 3.8.2).
25 Impaired glucose metabolism (diabetes mellitus) has been observed in patients with
26 pancreatic cancer, although it is not known whether diabetes develops shortly before or
27 after the clinical manifestations of pancreatic cancer. A meta-analysis of 11 case-control
28 studies (including only studies in which diabetes was present at least 1 year before
29 diagnosis of pancreatic cancer) and 9 cohort studies found a relative risk of 2.1 (95% CI
30 = 1.6 to 2.8) for pancreatic cancer in diabetics, compared with non-diabetics (Everhart
31 and Wright 1995). A large prospective cohort study found an association between post-

1 load plasma glucose concentration and pancreatic cancer in individuals without self-
2 reported diabetes, which the authors considered suggestive that factors associated with
3 abnormal glucose metabolism could play a role in development of pancreatic cancer
4 (Gapstur *et al.* 2000). In a review of pancreatic cancer, Michaud (2004) concluded that
5 chronic pancreatitis and diabetes mellitus are medical conditions that have been
6 consistently related to pancreatic cancer, and the evidence suggests that they are
7 causally related to pancreatic cancer rather than consequences of the cancer.

8 Matanoski and Tao (2003) reported an association between cardiovascular disease and
9 occupational exposure to styrene. Their case-cohort study included 498 cases that died
10 from ischemic heart disease and a 15% random sample (N = 997) of all male workers
11 who were employed during 1943 to 1982 in two styrene-butadiene rubber manufacturing
12 plants in the United States. Recent styrene exposure was significantly associated with
13 acute ischemic heart disease death among active workers. The relative hazard of death for
14 exposure during the most recent two years among active workers with 2 or more years of
15 employment was 2.95 (95% CI = 1.02 to 8.57) at a time-weighted styrene concentration
16 of 0.2 to < 0.3 ppm and 4.3 (95% CI = 1.56 to 11.84) at time-weighted exposure
17 concentrations of ≥ 0.3 ppm. Delzell *et al.* (2005) also examined the relationship of
18 styrene exposure and mortality from ischemic heart disease among 16,579 men employed
19 at 6 styrene-butadiene rubber manufacturing plants (including the 2 plants reported by
20 Matanoski and Tao) for at least one year and employed from 1943 until 1990. Men in the
21 highest quintile of exposure (> 5.5 ppm) and in the highest quintile of cumulative
22 exposure (> 60.67 ppm-year) had ischemic heart disease ratios of 1.14 (95% CI = 0.96 to
23 1.35), and 1.06 (95% CI = 0.90 to 1.27), respectively. Acute disease was not associated
24 with average intensity of exposure within the most recent 2 years. Incidences of chronic
25 disease were elevated in subjects with the highest exposure, but the associations were
26 weak and imprecise, and there was limited evidence of a dose-response relationship. The
27 authors concluded that their study did not provide strong support for a causal association
28 between styrene and mortality from ischemic heart disease.

29 The potential reproductive and developmental effects of styrene in humans have been
30 reviewed (IARC 2002, NTP 2006). Some earlier studies suggested an association

1 between occupational exposure to styrene and congenital CNS malformation and
2 spontaneous abortions; however, these associations were not confirmed in later studies
3 (IARC 2002). Other studies have not shown a consistent or statistically significant
4 relationship between styrene exposure and reduced birth weight, sperm abnormalities,
5 time-to-pregnancy, or menstrual cycle effects. NTP (2006) concluded that the human data
6 were insufficient to conclude that styrene is a reproductive or developmental toxicant;
7 however, based on the animal data, the panel expressed negligible concern for effects in
8 humans. There was suggestive evidence that occupational exposure to styrene was
9 associated with increased serum prolactin and depletion of peripheral blood dopamine-
10 metabolizing enzyme activities, but the clinical relevance of these findings was unclear.

11 Several publications have reported increased serum prolactin levels in workers exposed
12 occupationally to styrene (Arfini *et al.* 1987, Bergamaschi *et al.* 1996, Bergamaschi *et al.*
13 1997, Mutti *et al.* 1984); a proposed cause is dopaminergic dysfunction resulting from the
14 interaction between styrene metabolites and dopamine (Mutti and Smargiassi 1998).

15 Although the relationship between the possible styrene-related increases in serum
16 prolactin and breast cancer is not known, Harvey (2005) concluded that the evidence for
17 the role of prolactin in human breast cancer is strong and consistent. Several large
18 epidemiology studies have shown that dopamine antagonists increase breast cancer risk.
19 Hyperprolactinemia is associated with human breast cancer growth and poor prognosis,
20 and prolactin is a mitogen in human breast cancer cells that suppresses apoptosis and
21 upregulates BRCA1. An increased risk of breast cancer was not observed in the cohort
22 studies of styrene-exposed workers, which consisted predominantly of men; however,
23 two studies of the general population (a case-control study and an ecological study)
24 reported an association between breast cancer and styrene exposure (see Sections 3.5.2
25 and 3.8.6).

26 5.2.2 *Experimental animals*

27 The toxic effects of styrene in experimental animals have been reviewed (ATSDR 1992,
28 Bond 1989, IARC 2002). Acute exposures were associated with eye and nose irritation,
29 CNS depression, and death at high concentrations. Subacute to subchronic exposures
30 have been associated with adverse effects on the liver, pancreas, kidney, nervous system,

1 respiratory system, immune system, and hematopoietic system. This section briefly
2 reviews the overall toxicity in experimental animals (5.2.2.1) and then describes in
3 greater detail studies of respiratory toxicity (5.2.2.2), toxicity of the stereoisomers of
4 styrene-7,8-oxide (5.2.2.3), and glutathione depletion (5.2.2.4), because these factors
5 have been suggested to play a role in the development of lung tumors in mice (see
6 Sections 5.3 and 5.5).

7 5.2.2.1 Overall toxicity findings

8 Acute exposures of rats and guinea-pigs to styrene at a concentration of 650 ppm resulted
9 in eye and nose irritation (IARC 2002). Higher concentrations resulted in weakness,
10 unsteadiness, and other CNS effects (1,300 ppm), unconsciousness (2,500 ppm) and
11 death (5,000 to 10,000 ppm). Eye and nose irritation was also reported in rats exposed to
12 styrene concentrations of 1,300 or 2,000 ppm for 7 to 8 hours per day, 5 days per week
13 for about 6 months (Spencer *et al.* 1942, Wolf *et al.* 1956).

14 Permanent hearing loss, neurotoxic effects, hematopoietic and immune system effects,
15 and damage to the pancreas, lung, liver, and kidney have been reported in rats, mice, or
16 guinea-pigs (IARC 2002). Sliwinska-Kowalska *et al.* (2007) reported that the lowest
17 concentration of styrene known to cause hearing loss in rats is 300 ppm. Styrene damages
18 the outer hair cells in the cochlea. The neurotoxic effects included decreased monoamine
19 oxidase activity, depletion of dopamine, weakness, and brain damage. Gagnaire *et al.*
20 (2006) investigated the effects of styrene on the extracellular and tissue levels of
21 dopamine, serotonin, and their metabolites in male rats exposed to 750- or 1,000-ppm
22 styrene for 4 weeks. Rats exposed to the high dose had a significant decrease in
23 extracellular acid metabolite concentrations, while tissue levels of these metabolites were
24 decreased to a lesser extent. The effects were reversed after 17 days. Umemura *et al.*
25 (2005) investigated the neuroendocrinological effects in rats exposed to 150-ppm styrene
26 for 10 days. The styrene concentration in the blood was higher in female rats than in male
27 rats, and the prolactin level was significantly increased in female rats. Levels of
28 neurotransmitters were not affected in either sex; therefore, the mechanism enhancing
29 prolactin secretion was unclear. Mouse splenic T-lymphocyte activity was suppressed by
30 *in vitro* exposure to styrene, and oral dosing (20 to 50 mg/kg b.w. styrene daily for five

1 days) impaired humoral and cell-mediated immunity in male Swiss mice (IARC 2002).
2 The number of erythropoietic cells was increased in male Sprague-Dawley rats exposed
3 to styrene by inhalation or i.p. injection. Nano *et al.* (2000) exposed groups of 6 male
4 Sprague-Dawley rats to i.p. injections of styrene at 40 or 400 mg/kg b.w. or corn oil for 3
5 consecutive days or by inhalation of styrene vapor (purity 99%) at 0 or 300 ppm 6
6 hours/day, 5 days/week for 2 weeks. Some of the rats (inhalation exposure) were killed
7 immediately after the last treatment while the others were killed 3 weeks later. Rats
8 injected with 400 mg/kg styrene showed hyperactivity of the erythropoietic series while
9 the granulocytopoietic series was normal. There was a statistically significant increase in
10 basophilic, polychromatophilic, and orthochromatic erythroblasts in rats that inhaled
11 styrene for 2 weeks. There also was a temporary block of immature cells of the
12 granulocytopoietic series.

13 Khanna *et al.* (1994) exposed mice, rats, and guinea-pigs to styrene orally in groundnut
14 oil on 5 days per week for 4 weeks at 25 or 50 mg/kg b.w. per day for mice and 160 or
15 320 mg/kg b.w. per day for rats and guinea-pigs. Mice exhibited moderate inflammatory
16 reaction of pancreatic islet cells, congestion of pancreatic blood vessels, moderate
17 congestion of pancreatic lobules, and increased serum insulin levels. Guinea-pigs showed
18 congestion of pancreatic acinar parenchyma, marked degranulation of beta cells of large
19 pancreatic islets, and decreased serum insulin levels. No changes in the pancreas were
20 noted in rats other than decreased serum insulin levels, and no significant changes in
21 blood glucose levels were noted in any of the species studied.

22 Subacute to subchronic exposure to styrene by i.p. injection or inhalation has caused
23 kidney and liver damage in rodents (IARC 2002). These effects were often associated
24 with glutathione depletion. B6C3F₁ mice exposed for 14 days to styrene by inhalation at
25 a concentration of 0, 125, 250, or 500 ppm developed severe centrilobular hepatic
26 necrosis (Morgan *et al.* 1993c, Morgan *et al.* 1993b). Mortality was higher in the 250-
27 ppm group of each sex than in the 500-ppm group. The differences in mortality could not
28 be explained on the basis of styrene-7,8-oxide production, GSH depletion, or
29 hepatotoxicity. Sprague-Dawley rats given repeated i.p. injections of 2.9 to 5.8 mg/kg
30 b.w. for 6 weeks had morphological changes in the kidney (IARC 2002). Mild tubular

1 damage occurred in Sprague-Dawley rats given daily i.p. injections of 1 g/kg b.w. for 10
2 days, and inhalation exposure to 300 ppm for 12 weeks resulted in a slight increase in the
3 urinary excretion of plasma proteins and minor changes in kidney histopathology.
4 Hepatotoxic effects included focal necrosis in male albino rats exposed orally to 400
5 mg/kg b.w. styrene for 100 days. Centrilobular necrosis was reported in several studies in
6 mice exposed to 125 to 500 ppm for 2 weeks. Sex and strain differences in sensitivity
7 have not generally correlated with differences in glutathione depletion or concentrations
8 of styrene or styrene-7,8-oxide in blood. Single i.p. injections of 250 to 1,000 mg/kg b.w.
9 of styrene or styrene-7,8-oxide produced a dose-dependent increase in serum sorbitol
10 dehydrogenase activity [an indicator of hepatotoxicity]. One study indicated that liver
11 toxicity was greater when styrene was administered by i.p. injection compared with
12 inhalation of styrene vapor (De Piceis Polver *et al.* 2003). This may be explained by the
13 fact that the intraperitoneal route results in direct exposure of the liver. Several studies
14 have shown that hepatotoxicity may be enhanced in mice pretreated with CYP enzyme
15 inducers. In one study, the hepatotoxic effects of styrene-7,8-oxide were increased in
16 mice by administration of trichloropropene oxide, an inhibitor of epoxide hydrolase.

17 The developmental and reproductive toxicity of styrene in experimental animals have
18 been reviewed (Brown *et al.* 2000, IARC 2002, NTP 2006). The available studies have
19 not shown an increased incidence of malformations, but there have been reports of
20 increased embryonic, fetal, and neonatal deaths; skeletal and kidney abnormalities;
21 decreased birth weight, postnatal developmental delays (e.g., incisor eruption, eye
22 opening), and neurobehavioral and neurochemical abnormalities. The reported effects
23 were seen mostly at high doses that were associated with maternal toxicity, but at least
24 one study indicated that styrene might affect the developing brain and postnatal
25 development. Beliles *et al.* (1985) conducted a three-generation study of the reproductive
26 effects of styrene exposure in Sprague-Dawley rats (see Section 4.2.1). The animals were
27 exposed to styrene indirectly *in utero* and as neonates, and directly in drinking water
28 while maturing to become breeders. Fertility, litter size, pup viability, pup survival, sex
29 ratio, pup body weight, weanling kidney and liver weight, and marrow cytogenetics were
30 evaluated. The only reported effects included an apparent reduction in 21-day survival of
31 high-dose F₁ pups, and a loss of breeding efficiency in F₃ parents; however, the authors

1 noted that there were mitigating factors. These effects were not consistent and were
2 traced to a single or only two individual animals or litters. The authors concluded that
3 styrene exposure produced no deleterious dose-related effects or decrements in
4 reproductive function through three generations.

5 5.2.2.2 *Respiratory toxicity*

6 Respiratory toxicity, including nasal toxicity and pneumotoxicity, has been observed in
7 mice exposed to styrene or styrene-7,8-oxide. The Clara cell is the main site of both
8 bioactivation and toxicity of styrene in the lung (Harvilchuck and Carlson 2006, Hynes *et*
9 *al.* 1999).

10 Green *et al.* (2001b, 2001a) exposed CD-1 mice to styrene at 40 or 160 ppm for 3 days.
11 Styrene exposure caused degenerative changes in the nasal cells (including atrophy of the
12 olfactory mucosa and loss of normal cellular organization) and pneumotoxicity
13 (characterized by focal loss of cytoplasm and focal crowding of nonciliated Clara cells,
14 particularly in the terminal bronchiolar region). In mice exposed for 3 days or longer, cell
15 replication rates were increased in the terminal and large bronchioles (Green *et al.*
16 2001a). Similar effects occurred in mice given oral doses of 100 or 200 mg/kg for 5 days,
17 but not in rats exposed to 500 ppm for up to 10 days. There were no morphological or
18 cell proliferation effects in the alveolar region of the mouse lung. Female CD-1 mice
19 exposed to styrene at 40 or 160 ppm for 1 to 20 consecutive days had decreased levels of
20 Clara-cell-specific protein (CC16) in lung lavage fluid and blood serum, suggesting
21 destruction of Clara cells (Gamer *et al.* 2004). Swiss-Albino mice given i.p. injections of
22 styrene at 800 mg/kg b.w. or styrene-7,8-oxide at 300 mg/kg b.w. had increased levels of
23 gamma-glutamyltranspeptidase (GGT) and lactate dehydrogenase (LDH) in the
24 bronchioalveolar lavage fluid [these are markers of pneumotoxicity] (Gadberry *et al.*
25 1996).

26 Cruzan *et al.* (1997) reported nasal toxicity (atrophy of the olfactory epithelium and
27 olfactory nerve fibers, with or without focal respiratory metaplasia) and lung toxicity in
28 CD-1 mice exposed to styrene at a concentration of 100, 150, or 200 ppm for 13 weeks;
29 at 50 ppm, only nasal toxicity was seen. Changes in the lung included decreased
30 eosinophilia of the bronchial epithelium, focal crowding of nonciliated cells in

1 bronchioles, and focal bronchiolar epithelial proliferation. An increased labeling index in
2 Clara cells was observed after two weeks, but no increase was observed in type II
3 pneumocytes; however, the authors reported that the labeling index in the cell
4 proliferation studies was highly variable among rodents in the same exposure group.

5 In the chronic inhalation study (see Section 4.1.2), styrene exposure (20, 40, 80, or 160
6 ppm) resulted in toxic effects in the nasal passages and lung of CD-1 mice (Cruzan *et al.*
7 2001). Histological effects in the nasal passages included respiratory metaplasia of the
8 olfactory epithelium with changes in the underlying Bowman's glands and loss of
9 olfactory nerve fibers. The effects increased in severity with increasing styrene
10 concentration and duration of exposure, and most changes were observed in all exposure
11 groups by 78 weeks. In the lung, styrene exposure resulted in decreased eosinophilic
12 staining of Clara cells at 12, 18, and 24 months. Bronchiolar epithelial hyperplasia was
13 observed at 12 months (at concentrations > 40 ppm) or 18 months (at 20 ppm); the
14 hyperplasia extended into the alveolar ducts in the high-dose animals. (Lung tumors were
15 observed after 24 months, see Section 4.1.2)

16 Respiratory toxicity has also been reported in rats exposed to styrene. Exposure at 150 or
17 1,000 ppm caused a dose-related decrease in tracheal and nasal ciliary activity, but at 12
18 weeks post exposure, ciliary activity had returned to near control values in the low-dose
19 group and to 50% to 75% of control values in the high-dose group (Ohashi *et al.* 1986).
20 Epithelial changes in the nose and trachea (vacuolation of epithelial cells, nuclear
21 pyknosis, and exfoliation of epithelial cells) were observed in rats exposed to styrene at
22 800 ppm (IARC 2002). Cruzan *et al.* (1997) also reported histological changes in the
23 olfactory epithelium (focal disorganization, focal hyperplasia of basal cells, single-cell
24 necrosis, and cell loss) in CD (Sprague-Dawley-derived) rats exposed to styrene by
25 inhalation at 500 to 1,500 ppm for 13 weeks. Coccini *et al.* (1997) reported cytoplasmic
26 changes involving bronchiolar and alveolar type II cells (similar to those observed in
27 mice) in Sprague-Dawley rats exposed to styrene by either i.p. injection (40 or 400 mg/kg
28 b.w. daily) or inhalation (300 ppm for 2 weeks); damage was more severe following i.p.
29 injection.

1 In contrast, several studies have not detected pneumotoxicity in rats. Gamer *et al.* (2004)
2 reported no signs of lung toxicity in female CD rats exposed to styrene at up to
3 2,150 mg/m³ [490 ppm] for up to 21 days, and Green *et al.* (2001a) did not observe
4 morphological or cell-proliferative changes in the lungs of Sprague-Dawley rats exposed
5 at 500 ppm for up to 10 days; however, lung toxicity was observed in mice in these
6 studies (see above). Cruzan *et al.* (1997) also did not observe lung toxicity or increased
7 cell proliferation in CD rats [although there was a high variability in the percentage of
8 cells labeled with bromodeoxyuridine].

9 Some studies have suggested that styrene metabolites other than styrene-7,8-oxide also
10 cause cytotoxicity in the lung. Cruzan *et al.* (2002) reported that styrene metabolism in
11 mice produced 4- to 10-fold more metabolites via ring-oxidation and the
12 phenylacetaldehyde pathways than observed in rats. In another study, the toxicity of 4-
13 vinylphenol, a ring-oxidized metabolite of styrene, was evaluated in lungs of CD-1 mice
14 and female Sprague-Dawley rats exposed by i.p. injection in 3 daily divided doses (2, 6,
15 20, or 60 mg/kg b.w. per day) for 14 consecutive days (Cruzan *et al.* 2005a). Multifocal
16 hyperplasia was present in the medium bronchi and terminal bronchioles in some of the
17 mice exposed to 6 or 20 mg/kg b.w. and in all of the mice in the high-dose group.
18 However, no evidence of toxicity was found in the lungs of Sprague-Dawley rats. Several
19 studies have investigated the metabolism of the styrene metabolite 4-vinylphenol in rat
20 and mouse liver and lung. Carlson *et al.* (2002) concluded that 4-vinylphenol is a more
21 potent hepatotoxicant and pneumotoxicant than either styrene or styrene-7,8-oxide based
22 on increases in SDH (a marker for hepatic toxicity) in serum and increases in cell
23 numbers and LDH levels in bronchoalveolar lavage fluid from adult male CD-1 mice
24 injected i.p. with 50 mg/kg 4-vinylphenol compared with doses of 500 to 1,000 mg/kg for
25 styrene and 300 mg/kg styrene oxide to induce significant effects in separate experiments
26 from their laboratory in another strain of mice (non-Swiss Albino) (Gadsberry *et al.*
27 1996).

28 Kaufmann *et al.* (2005) investigated the effects of styrene and its metabolites on the
29 mouse lung. CD-1 mice were injected i.p. with styrene, styrene-7,8-oxide, 4-vinylphenol,
30 1-phenylethanol, 2-phenylethanol, phenylacetaldehyde, phenylacetic acid, or

1 acetophenone. Of the compounds tested, only styrene-7,8-oxide (at 100 mg/kg b.w. 3
2 times per day) and 4-vinylphenol (5, 20, or 35 mg/kg b.w. 3 times per day) caused
3 increases in cell proliferation in large/medium bronchi (up to 15.1 fold for 4-vinylphenol
4 and 7.5 fold for styrene-7,8-oxide) and terminal bronchioles (up to 19.7 fold for 4-
5 vinylphenol and 10.5 fold for styrene-7,8-oxide). Both compounds also caused
6 glutathione depletion and histomorphological changes in the bronchiolar epithelium.
7 These two molecules also caused histopathological changes in the terminal bronchioles
8 that included the appearance of flattened cells and the loss of the typical bulging of the
9 apical cytoplasm of Clara cells (which the authors describe as “dome-shaped”) into the
10 bronchial lumina. Styrene-7,8-oxide, but not 4-vinylphenol, also caused marginal
11 increases in alveolar cell proliferation and an increased number of apoptotic cells in
12 large/medium bronchi. Kaufmann *et al.* concluded that the metabolites of the side-chain
13 hydroxylation pathway (phenylethanols, acetophenone, phenylacetaldehyde, and
14 phenylacetic acid) were of minor relevance for the pneumotoxic effects in the terminal
15 bronchioles, and they proposed that ring-oxidized metabolites could be the cause of
16 styrene-induced oncogenicity based on the cytotoxicity of the ring-oxidized metabolite 4-
17 vinylphenol for Clara cells and the resulting proliferative response in the terminal
18 bronchioles.

19 Chung *et al.* (2006) compared the cytotoxicity of styrene and styrene-7,8-oxide in a
20 transgenic cell line expressing CYP2E1 (h2E1) and the wild-type cell line (cHol, human
21 B-lymphoblastoid). Cell viability assays demonstrated that styrene was toxic to h2E1
22 cells ($IC_{50} = 121.8 \mu\text{M}$) but no significant increase in cell death was observed in wild-
23 type cells at concentrations as high as 1,000 μM . However, there was no significant
24 difference in susceptibility of h2E1 and wild-type cells exposed to styrene-7,8-oxide.
25 These data indicate that CYP2E1 and styrene-7,8-oxide have an important role in the
26 cytotoxic effects of styrene in these cell lines. Inhibition of epoxide hydrolases enhanced
27 cytotoxicity while glutathione conjugation reduced cytotoxicity.

28 5.2.2.3 Toxicity of styrene stereoisomers

29 Gadberry *et al.* (1996) examined the pneumotoxicity and hepatotoxicity of styrene and
30 styrene-7,8-oxide (the racemic mixture and *R*- and *S*-enantiomers) in adult male non-

1 Swiss albino mice. GGT and LDH activity in bronchioalveolar lavage fluid and the
2 activity of the hepatic enzyme serum sorbitol dehydrogenase (SDH) were measured.
3 Groups of 8 to 10 mice were sacrificed 24 hours after i.p. injection with 300 mg/kg b.w.
4 of either racemic, *R*-, or *S*-styrene-7,8-oxide or 800 mg/kg b.w. of styrene, and another
5 group of mice was sacrificed 6 hours after i.p. injection with 300 mg/kg b.w. of either
6 racemic, *R*-, or *S*-styrene-7,8-oxide. Data for the 24-hour sacrifice are shown in Figure 5-
7 2. The *R*-isomer of styrene-7,8-oxide was more hepatotoxic than the *S*-isomer at both the
8 6-hour (data not shown) and 24-hour time points, based on a significant ($P < 0.05$)
9 increase in SDH activity. In the tests for pneumotoxicity (GGT and LDH), enzyme
10 activity was higher at both time points in the lungs of mice administered the *R*-isomer
11 than in those administered the *S*-isomer. The results for the racemic mixture were
12 variable, being sometimes higher than for either individual isomer, sometimes lower, and
13 sometimes intermediate. However, the authors reported that none of the differences were
14 statistically significant.

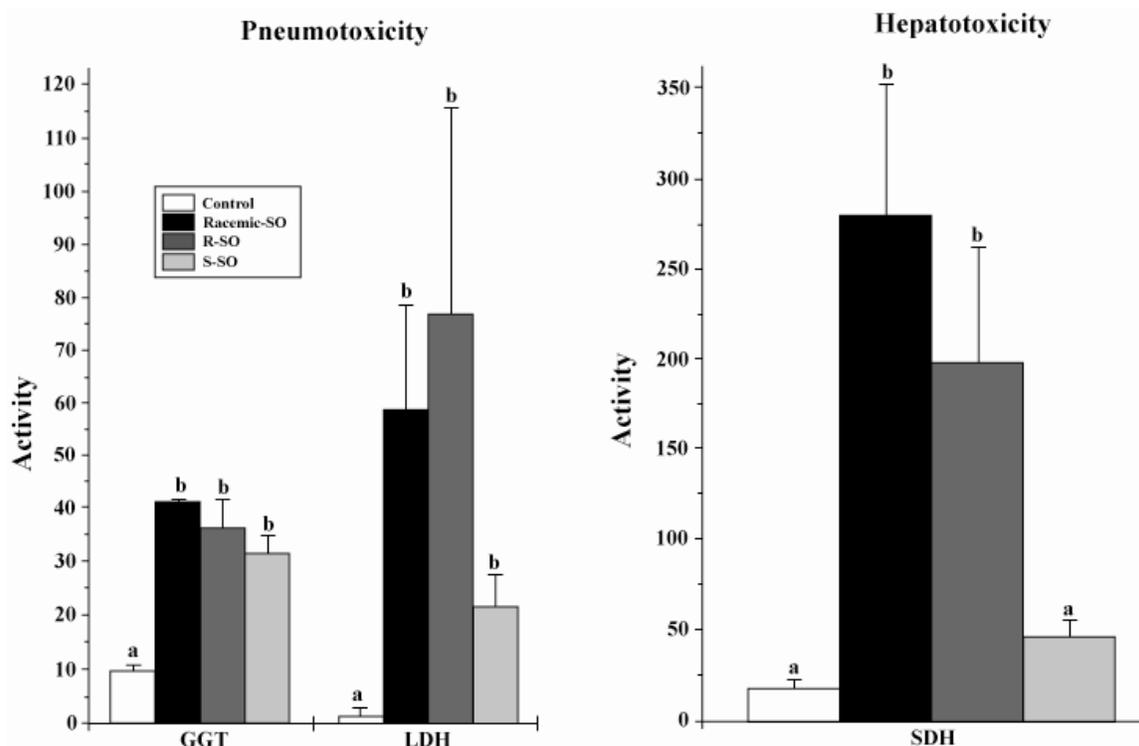


Figure 5-2. Pneumotoxicity and hepatotoxicity of styrene-7,8-oxide enantiomers in male non-Swiss albino mice at 24 hours after i.p. administration

Source: adapted from Gadberry *et al.* 1996.

Results for exposure groups with different letters (a, b) differed significantly from each other at $P < 0.05$.

1 In an *in vitro* study using isolated Clara-cell (~90% of total cells) preparations prepared
2 from lungs of adult male CD-1 mice, Harvilchuck and Carlson (2006) reported that
3 styrene (LC₅₀ = 1.721 mM) was the most toxic substance tested, followed by racemic
4 styrene-7,8-oxide (2.344 mM), *R*-styrene-7,8-oxide (3.243 mM), 4-vinylphenol (3.500
5 mM), and *S*-styrene-7,8-oxide (4.842 mM); [however, no statistical comparisons were
6 reported and the toxicity data for the various compounds appeared to overlap in the
7 published graph]. Clara cells isolated from Sprague-Dawley rats were 4-fold less
8 susceptible to cytotoxicity of styrene and its metabolites than mouse Clara cells. Styrene
9 also was the most toxic compound tested in isolated rat Clara cells with 4-vinylphenol
10 and *S*-styrene-7,8-oxide being the least toxic. Incubation of mouse Clara cells with test
11 agents at 0.1, 0.5, and 1.0 mM concentrations resulted in significantly ($P < 0.05$, Student-
12 Newman-Keul's test) decreased glutathione levels after a 3-hour incubation with the
13 following substances and concentrations: styrene (1.0 mM), racemic styrene-7,8-oxide
14 (0.5 or 1.0 mM), *R*-styrene-7,8-oxide (1.0 mM), *S*-styrene-7,8-oxide (1.0 mM), and 4-
15 vinylphenol (0.5 or 1.0 mM). In *in vivo* experiments, racemic and *R*-styrene-7,8-oxide
16 (300 mg/kg b.w. doses for each) significantly ($P < 0.05$, Student-Newman-Keul's test)
17 decreased Clara-cell glutathione concentrations at 3 hours after intraperitoneal injection
18 of test agents compared with corn-oil controls, while neither styrene (600 mg/kg b.w.), *S*-
19 styrene-7,8-oxide (300 mg/kg b.w.), nor 4-vinylphenol (100 mg/kg b.w.) differed
20 significantly from controls at this time point.

21 Several studies have shown differential genotoxicity of the styrene-7,8-oxide
22 enantiomers, in the following order of mutagenicity: *R*-enantiomer > racemic mixture >
23 *S*-enantiomer (Pagano *et al.* 1982, Seiler 1990). However, chromosomal aberrations and
24 sister chromatid exchange in mouse bone marrow cells were increased significantly
25 following *in vivo* exposure to the *S*-isomer but not the *R*-isomer, and the mitotic index
26 was decreased significantly for both isomers (Sinsheimer *et al.* 1993). (See Section
27 5.5.2.4 for a more detailed discussion of these studies).

28 5.2.2.4 Glutathione depletion

29 Depletion of GSH in the lung has been reported to be associated with an increased risk of
30 lung damage and disease (Rahman *et al.* 1999), and GSH depletion generally has been

1 shown to correlate with chromosomal DNA fragmentation associated with apoptosis and
2 necrosis (Higuchi 2004). Although Cohen *et al.* (2002)⁶ reported that both measured
3 GSH concentrations and those predicted by a physiologically based pharmacokinetic
4 (PBPK) model indicated a greater decrease in GSH in the lungs of mice than rats, these
5 effects were seen only at styrene concentrations of 40 ppm or greater, which exceeded the
6 concentration of 20 ppm at which hyperplasia in the mouse lung was reported by Cruzan
7 *et al.* (2001). Also, Gamer *et al.* (2004) reported that 20 exposures (6 hours per day, 5
8 consecutive days per week, for 4 weeks) of female CD-1 mice to styrene at 160 ppm,
9 which produced cellular crowding in the epithelial lining of the lung, indicative of very
10 early hyperplasia, did not increase concentrations of 8-hydroxy-deoxyguanosine. No
11 evidence of oxidative stress was seen despite depletion of GSH in homogenates from the
12 styrene-exposed mouse lungs. Similarly exposed female Crl:CD rats did not show any
13 signs of lung toxicity. Turner *et al.* (2005) also found a decrease in GSH in the lungs of
14 mice exposed by i.p. injection to styrene (600 mg/kg b.w., or 5.8 mmol/kg) and styrene-
15 7,8-oxide (300 mg/kg b.w., or 2.5 mmol/kg). However, administration of 4-vinylphenol
16 (100 mg/kg, or 0.83 mmol/kg), which is a more potent hepatotoxin and pneumotoxin than
17 styrene or styrene-7,8-oxide, caused less depletion of GSH.

18 5.2.3 Estrogenicity studies

19 Several studies have been published on the potential estrogenicity of polystyrene
20 oligomers, which can leach from polystyrene food containers. Polystyrene dimer and
21 trimer extracts from food containers were tested *in vitro* for estrogen-like effects using
22 estrogen-responsive element reporter, estrogen receptor binding, and cell-proliferation
23 assays, and *in vivo* using a rat uterotrophic assay. Bachman *et al.* (1998) measured the
24 effect of extracts from 23 polystyrenes in a rat uterotrophic assay at concentrations up to
25 0.75 mg/L [equivalent to 15 microgram/kg-b.w. per day]. None of the polystyrene
26 extracts were positive in this assay. Fail *et al.* (1998) measured the estrogenicity of a
27 polystyrene extract equivalent in dose per body weight to human consumption [amount
28 not specified]. It was negative in a rat uterotrophic assay and in an estrogen-responsive
29 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).

1 extract tested]. Azuma *et al.* (2000) and Date *et al.* (2002) reported a lack of estrogenicity
2 of styrene monomers, dimers and trimers using *in vivo* and *in vitro* assay systems. Ohno
3 *et al.* (2001) used high concentrations [up to 10^{-3} M *in vitro*] of specific oligomers in
4 uterotrophic, estrogen-responsive element reporter, and estrogen receptor binding assays
5 and also obtained negative results. In this study, styrene monomer, three styrene dimers,
6 and seven styrene trimers known to dissolve in small amounts from polystyrene cup
7 noodle containers were tested. However, Ohyama *et al.* (2001) tested the same styrene
8 dimers and trimers and obtained positive results (2 positives out of 4 dimers tested and 4
9 positives out of 7 trimers tested) at concentrations of 10^{-6} and 10^{-5} M [highest
10 concentration tested] in a cell-proliferation assay and in a binding affinity assay for
11 human estrogen receptor alpha (9 oligomers were positive and 2 trimers were negative in
12 this assay). These results were refuted by Ohno *et al.* (2003), whose laboratory tested the
13 same oligomers from the Ohyama report using three different estrogen receptor binding
14 assays. The results for all oligomers were negative in these assays. Further, the results of
15 a rat uterotrophic assay and estrogen response element reporter assay were also negative
16 using the same styrene oligomers. In a letter to the journal *Environmental Health*
17 *Perspectives*, Ohno and colleagues (Ohno *et al.* 2002) noted that in the assay system of
18 Ohyama, solubility was a problem at high concentrations leading to false positive results,
19 and the validity of the MCF-7 E-Screen assay was also questioned. Ohyama and Nagi
20 replied that their results were valid, because they believed the insolubility of styrene
21 oligomers observed in Ohno and colleagues' studies appeared to be due to using water
22 rather than DMSO (as was used in the Ohyama studies) to dissolve the compounds.
23 Ohyama also defended the use of the MCF-7 E-screen method as a well recognized
24 method for estrogenic screening.

25 It is possible that metabolic activation of styrene oligomers may affect the estrogenicity
26 of these compounds. Kitamura *et al.* (2003), using rat liver microsomes as a metabolic
27 activating system, found the activated form of *trans*-1,2 diphenylcyclobutane, a styrene
28 dimer, to be estrogenic using a yeast estrogen screening assay and an estrogen-responsive
29 element reporter assay. The active metabolite was a hydroxylated form called *trans*-1(4-
30 hydroxyphenyl)2-phenylcyclobutane [activity at 10^{-5} M]. According to the authors, *cis*-
31 1,2-diphenylcyclobutane, 1,3-diphenylpropane, and 2, 4-diphenyl-1-butene also exhibited

1 estrogenic activity after metabolic activation, but the activity was lower than with *cis*-1,2-
2 diphenylcyclobutane.

3 **5.3 Interspecies differences in metabolism, toxicity, and toxicokinetics**

4 In its summary of styrene exposure studies in humans (volunteers or workers) and
5 experimental animals, IARC (2002) stated that toxicokinetic pathways are qualitatively
6 similar in humans and animals, but differ quantitatively. This section reviews studies and
7 toxicokinetic models of interspecies differences in styrene-7,8-oxide formation,
8 stereochemistry, and metabolism of styrene-7,8-oxide.

9 *5.3.1 Styrene-7,8-oxide formation in the lung*

10 Styrene-7,8-oxide, a primary metabolite of styrene, is considered to cause many of the
11 toxic and genotoxic effects resulting from styrene exposure, including those in the lung.
12 Clara cells are primarily responsible for the metabolism of styrene to styrene-7,8-oxide in
13 the lung (see Section 5.1.3), and metabolism results in formation of two optically active
14 enantiomers (*R*- and *S*-forms) because of the chiral carbon in styrene-7,8-oxide. Plopper
15 *et al.* (1980a, 1980b) identified interspecies morphological differences in Clara cells that
16 are consistent with the observed differences in styrene metabolism in rodent and human
17 lung. Mouse and rat Clara cells contain an abundance of agranular endoplasmic
18 reticulum, which is associated with metabolism of pulmonary toxins via cytochromes
19 P450. Human Clara cells contain abundant granular endoplasmic reticulum but no
20 agranular endoplasmic reticulum.

21 Cohen *et al.* (2002) reviewed studies measuring conversion of styrene to styrene-7,8-
22 oxide by cytochrome P450 monooxygenase in pulmonary tissues in rats, mice, and
23 humans. Most studies showed styrene-7,8-oxide production to be highest in mice (0.95 to
24 4.5 nmol/min per mg protein), followed by rats (0.32 to 11.7 nmol/min per mg protein),
25 and humans (0.006 to 0.014 nmol/min per mg protein). Cohen *et al.* also noted that
26 metabolic conversion rates varied according to cell type or tissue. Mouse Clara cells
27 produced styrene-7,8-oxide (193 pmol/10⁶ Clara cells per minute) at 3 times the rate of
28 rat Clara cells (59 pmol/10⁶ cells per minute); however, when more aggregate pulmonary
29 tissue fractions (pulmonary microsomes) were compared, the rates differed by a factor of
30 1.5 (2.13 nmol/min per mg protein in mice vs. 1.44 in rats) (Hynes *et al.* 1999). However,

1 Cohen *et al.* concluded that differences in styrene-7,8-oxide concentrations in the lung do
2 not sufficiently explain the differences in susceptibility to the carcinogenic effects of
3 styrene between rats and mice. Rats did not develop lung tumors in groups that had
4 similar predicted styrene-7,8-oxide concentrations in the lungs compared with groups of
5 mice that developed lung tumors.

6 Hofmann *et al.* (2006) investigated the styrene-7,8-oxide levels formed in isolated lungs
7 of male Sprague-Dawley rats and *in-situ* prepared lungs from male B6C3F₁ mice. Styrene
8 vapor concentrations were measured in air samples collected in the immediate vicinity of
9 the trachea and were almost constant during each experiment. Styrene vapor
10 concentrations ranged from 50 to 980 ppm for rats and 40 to 410 ppm for mice. Both
11 species metabolized styrene to styrene-7,8-oxide; however, mean styrene-7,8-oxide levels
12 in mouse lungs were about 2 times higher than in rat lungs at equal exposure conditions.

13 5.3.2 Detoxification of styrene-7,8-oxide in respiratory tissue

14 Styrene-7,8-oxide is detoxified through hydrolysis mediated by mEH or conjugation with
15 glutathione mediated by GST. Cohen *et al.* (2002) summarized studies in rodents and
16 humans measuring the capacity of mEH in pulmonary tissue to detoxify styrene-7,8-
17 oxide. The metabolic conversion rates (in nanomoles per minute per milligram of protein)
18 for hydration of styrene-7,8-oxide by mEH to form styrene glycol varied among studies,
19 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
20 3.4 in humans. The estimated K_m s for hydrolysis from a previous study were 0.013 mM
21 in mice, 0.0047 mM in rats, and 0.0156 mM in humans. The metabolic conversion rates
22 for conjugation of styrene-7,8-oxide mediated by GST in pulmonary tissues varied
23 among studies (as summarized in Cohen *et al.* 2002); the one study in humans reported a
24 rate similar to that in one study in mice but lower than the rates from other studies in
25 mice or rats. The estimated ratio of V_{max} to K_m (which Cohen stated was an indication of
26 GST metabolic activity) was much lower in humans (19) compared with mice (171) or
27 rats (1,982).

28 Green *et al.* (2001b) investigated the cytochrome P450-mediated metabolism of styrene
29 to styrene-7,8-oxide and subsequent metabolism of styrene-7,8-oxide by either mEH or
30 GST in nasal and liver microsomal fractions from mice, rats, and humans. P450

1 metabolism of styrene to styrene-7,8-oxide was similar in rat and mouse olfactory and
2 respiratory fractions but was not detected in human nasal samples. Rates in rodent
3 olfactory fractions were higher than those measured in respiratory or liver fractions. The
4 rates of metabolism of *R* and *S* styrene-7,8-oxides via mEH in rat respiratory fractions
5 were up to 3.5-fold higher while rates in olfactory fractions were up to 10-fold higher
6 than in mice. Rates of mEH-mediated metabolism of styrene-7,8-oxide in human nasal
7 fractions were comparable with mouse olfactory and respiratory tissues and rat
8 respiratory tissues. Rodent nasal and respiratory tissues also metabolized styrene-7,8-
9 oxide via GST at rates significantly higher than those for mEH. Olfactory fractions from
10 rats had 3- to 4-fold greater rates of glutathione conjugation than observed in mice. In
11 contrast, metabolism of styrene-7,8-oxide by glutathione conjugation was undetectable in
12 5 of 6 samples of human nasal tissues, and the sixth sample metabolized styrene-7,8-
13 oxide at a much lower rate than did mouse or rat tissues.

14 5.3.3 Stereochemistry considerations

15 The metabolic activation of styrene to styrene-7,8-oxide enantiomers has been reported to
16 depend on tissue and species, and some authors have suggested that the *R*-enantiomer is
17 more toxic. Cohen *et al.* (2002) summarized the results of studies evaluating the ratio of
18 the *R*- to *S*-enantiomers of styrene-7,8-oxide produced through pulmonary and hepatic
19 metabolism. The results from these studies showed that mouse lung microsomes
20 produced greater amounts of the *R*-enantiomer than did microsomes from rat or human
21 lung. In mouse lung, the *R/S* ratio was usually between 2.4 and 2.6, although one study
22 reported a ratio of 1.7; in rat lung, the ratio was 0.52, based on one available study, and in
23 human lung, it was 1.15, based on 1 sample from one study. In hepatic microsomes, the
24 *R/S* ratio ranged from 1.18 to 1.78 in mice and was 0.57 in rats (one study) and 0.72 in
25 humans (one study). Cohen *et al.* reported that their PBPK model predicted that the *R/S*
26 ratio in mouse and rat lungs would be approximately twice as high as the ratio for total
27 styrene-7,8-oxide between the two species; nevertheless, these differences were
28 insufficient to explain the differences in susceptibility. In studies in mice, the *R/S* ratio
29 was 4.0 in isolated Clara cells, but 3.6 in type II cells. In rats, on the other hand, Clara
30 cells produced a nearly racemic mixture of enantiomers, and the *S*-isomer predominated

1 in type II cells. Green *et al.* (2001b) reported an *R/S* ratio of approximately 3 in nasal
2 tissue of rats and mice and liver tissue of mice, and a ratio of 0.72 in liver tissue of rats.

3 Linhart (2001) reported that human liver microsomes produced a nearly racemic mixture
4 of enantiomers; however, 2 samples showed a predominance of the *S*-isomer. Wenker *et*
5 *al.* (2001b) reported variable enantioselectivity in human liver microsomes, which
6 produced a moderate excess of the *S*-isomer at a low styrene concentration (16 μM)
7 (mean \pm SD = 14.7% \pm 6.9%) but an excess of the *R*-isomer at a high styrene
8 concentration (1,100 μM) (7.0% \pm 8.9%). When Wenker *et al.* (2000) compared the
9 metabolism of *R*- and *S*-styrene-7,8-oxide by 20 human liver microsomal preparations,
10 they found among the samples a 3- to 5-fold variation in the V_{max} , K_{m} , and $V_{\text{max}}/K_{\text{m}}$ values
11 for the two enantiomers. They were able to demonstrate that the mEH-mediated
12 hydrolysis of styrene-7,8-oxide favored hydrolysis of the reportedly more toxic *R*-
13 enantiomer, because the *S*-isomer had a higher K_{m} (by a factor of \sim 6) and V_{max} (by a
14 factor of \sim 5) than the *R*-isomer. The authors found no association between enzyme
15 kinetics and mEH polymorphisms at exons 3 and 4.

16 Because of the differences in enantiomeric excess found for each metabolite, Wenker *et*
17 *al.* (2001a) concluded that the individual enzymes responsible for the biotransformation
18 and excretion of styrene-7,8-oxide differed in their enantiomeric selectivity and/or
19 specificity. Hallier *et al.* (1995) determined that the *R/S* ratio ranged from 0.7 to 2.2 in 20
20 male German workers in the polyester industry exposed to styrene by inhalation at
21 concentrations ranging from 29 to 41 ppm; the differences in the *R/S* ratio could not be
22 explained by differences in individual exposure or in urinary metabolite concentrations.
23 The authors proposed that interindividual differences in metabolism of styrene to *R*- and
24 *S*-enantiomers were likely related to enzyme polymorphisms. Drummond *et al.* (1989)
25 measured excretion of *R*- and *S*-enantiomers of mandelic acid in three workers
26 occupationally exposed to 8-hour time-weighted average styrene concentrations of up to
27 420 mg/m^3 [100 ppm]. The *R/S* ratios for the three individuals were 1.16, 1.27, and 1.14.

28 Linhart (2001) reviewed the stereochemistry of styrene biotransformation and concluded
29 that the ratio of the enantiomers in a target tissue or cell will depend on both the

1 stereoselectivity of the formation of styrene-7,8-oxide and the stereoselectivity of the
2 metabolism of styrene-7,8-oxide. In rats, the formation reaction favors the *S*-enantiomer,
3 and detoxification of the *R*-enantiomer is faster. The formation reaction in mouse liver
4 and lungs favors the *R*-enantiomer, but detoxification moves the ratio closer to a racemic
5 mixture. Linhart concluded that the stereochemistry of styrene biotransformation might
6 contribute to species differences in toxicity between mice and rats but that it could not be
7 interpreted as a crucial factor. In addition, the author concluded that the relationship of
8 styrene stereochemistry to toxic effects in humans could not be interpreted, because
9 relevant data were lacking.

10 5.3.4 Kinetics of styrene metabolism and toxicokinetic models

11 In addition to the cell-specific metabolism of styrene discussed above, several studies
12 have focused on the kinetics of styrene and styrene-7,8-oxide metabolism in the whole
13 animal for several species (IARC 2002). In one study, the rate of metabolism of styrene
14 to styrene-7,8-oxide was compared among species: the order was guinea-pig > rabbit >
15 mouse > rat. However, for metabolism of styrene-7,8-oxide to styrene glycol by mEH,
16 the order was rat > rabbit > guinea-pig > mouse. In another study, the rate depended on
17 the styrene concentration, decreasing from mouse to rat to human at a low concentration,
18 but from rat to mouse to human at a high concentration (IARC 2002). Cruzan *et al.*
19 (2001, 1998) reported that styrene-7,8-oxide concentration in the blood was lower in
20 mice exposed to a concentration of 160 ppm [an exposure level associated with lung
21 cancer] than in rats exposed at 1,000 ppm [an exposure level at which no tumors were
22 observed].

23 Several pharmacokinetic models have been developed that compared styrene distribution
24 and metabolism in mice, rats, and humans. Sarangapani *et al.* (2002) reported that the
25 earlier models (Csanady *et al.* 1994, Ramsey and Andersen 1984) did not treat the
26 respiratory tract as a target organ and did not incorporate metabolic production and
27 clearance of styrene-7,8-oxide in the respiratory tract. Therefore, the Sarangapani *et al.*
28 PBPK model incorporated a multicompartmental description of the respiratory tract and
29 specifically added a compartment to represent the terminal bronchiolar region. This
30 model was based on metabolism of styrene in the liver and the terminal bronchiolar

1 region of the lung, which is richest in the metabolically active Clara cells and in which
2 the authors considered styrene-mediated toxicity to occur.

3 Filser *et al.* (2002) also developed a PBPK model for styrene in mice, rats, and humans
4 based on metabolism in both the liver and lung. This model divided the lung into two
5 compartments: the gas conducting zone and the gas exchange zone. The enzymatic
6 capacities of the two compartments were based on their shares of the total lung volume
7 because the kinetics of styrene and styrene-7,8-oxide metabolizing enzymes were
8 determined in microsomes and cytosol from whole lung tissue. They tested the validity of
9 their model by comparing the predicted area under the curve for blood styrene-7,8-oxide
10 concentration with reported values from published studies in Sprague-Dawley and F344
11 rats and B6C3F₁ mice.

12 Both of these models predicted that the order of styrene concentration in the lung (Filser
13 *et al.*) or terminal bronchioles of the lung (Sarangapani *et al.*) would be mouse > rat >
14 human. The Harvard Center for Risk Analysis also developed a PBPK model that
15 predicted the concentrations of styrene-7,8-oxide and *R*-styrene-7,8-oxide in the tissues
16 of humans, rats, and mice exposed to styrene at different concentrations (Cohen *et al.*
17 2002). This model used Csanády *et al.* (1994) as a starting point but included several
18 modifications (e.g., equations to account for styrene metabolism in the lung and to
19 estimate *R*- and *S*-styrene-7,8-oxide concentrations in various tissues). Results from this
20 model were inconclusive because of inconsistencies among studies in the measured levels
21 of styrene-7,8-oxide in the blood. Depending on the data used for calibration, the model
22 sometimes predicted higher concentrations of styrene-7,8-oxide in the lungs of rats, while
23 in other cases, higher concentrations were predicted for the mouse. However, Csanády *et*
24 *al.* (2003) reported that there was a typographical error in an equation described in
25 Csanády *et al.* 1994 that was overlooked by Cohen *et al.* (2002) and could explain their
26 inability to copy the Csanády *et al.* model.

27 Although the available data suggested that the mouse has a greater metabolic capacity for
28 converting styrene to styrene-7,8-oxide, and a greater pharmacokinetic response
29 (particularly with respect to lung tumors), the data were insufficient to explain why mice

1 were more susceptible than rats. These authors concluded that the existing
2 pharmacokinetic data failed to explain the observed differences in metabolite levels in
3 mice, rats, and humans. These authors further noted that the current inability to explain
4 species differences makes it difficult to determine whether the rat or the mouse is the
5 better model for the human response to styrene.

6 **5.4 Genetic and related effects**

7 Biotransformation of styrene to the genotoxic styrene-7,8-oxide seems to be responsible
8 for the majority of the genotoxic effects associated with styrene exposure (Cohen *et al.*
9 2002). Furthermore, during the manufacture of reinforced plastics, styrene and trace
10 amounts of styrene-7,8-oxide are released, thus, direct occupational exposure of workers
11 to styrene-7,8-oxide has been shown (see Section 2.5.1.6) (Nylander-French *et al.* 1999,
12 Rappaport *et al.* 1996, Tornero-Velez and Rappaport 2001). This section summarizes the
13 publicly-available peer-reviewed literature on the genetic and related effects of styrene.
14 Styrene genotoxicity has been investigated in many *in vitro* and *in vivo* studies and
15 reviewed in several publications (Barale 1991, Cohen *et al.* 2002, IARC 1994a, 2002,
16 Scott and Preston 1994a, Speit and Henderson 2005, Vodicka *et al.* 2006b). The genetic
17 and related effects discussed below include studies of DNA adducts, alkali-labile lesions,
18 DNA strand breaks, cytogenetic damage, and mutations, with a focus on mammalian
19 systems, especially human cells and studies of styrene-exposed workers.

20 *5.4.1 DNA adduct formation*

21 This section discusses formation and chemistry of styrene-7,8-oxide DNA adducts.
22 Specific studies of DNA adduct formation in cell cultures, experimental animals, and
23 styrene-exposed workers are discussed in the following sections.

24 DNA adduct detection methods are important tools for determining the etiology of human
25 cancer and for measuring metabolic enzyme and DNA repair system genotypes (Collins
26 1998, Hemminki *et al.* 2000, Perera and Weinstein 2000). The major metabolite of
27 styrene *in vivo* is styrene-7,8-oxide, which is expected to bind covalently to biological
28 macromolecules. The binding of styrene-7,8-oxide to nucleic acid constituents has been
29 studied extensively during the last 20 years; however, no studies on other styrene
30 metabolites with the potential to bind DNA (e.g., styrene 3,4-oxide) were identified.

1 Styrene-7,8-oxide possesses two sites (the α - and β -carbons of the epoxide moiety; see
2 Figure 1-2) that are electrophilic and able to react at nucleophilic sites in DNA. Either
3 carbon in the epoxide of styrene-7,8-oxide can react with nucleic acid, and because the
4 carbon atom at the 1-position is a chiral center, there are four possible diastereomers (*R*-
5 and *S*-isomers of the alpha form and *R*- and *S*- isomers of the beta form) (Phillips and
6 Farmer 1994).

7 The reaction mechanisms of styrene-7,8-oxide alkylation have been intensively studied
8 (Barlow and Dipple 1998, 1999, Latif *et al.* 1988, Qian and Dipple 1995). In this
9 document, binding sites for adducts are identified by the position of the atom as part of a
10 ring (the atom to which the adduct is bound is followed by its position in the ring [e.g.,
11 N3 of deoxyguanosine]) or as an exocyclic group on the ring (the atom to which the
12 adduct is bound is followed by the position of the ring atom to which it is bound,
13 superscripted [e.g., N² of deoxyguanosine]). The primary target of styrene-7,8-oxide
14 alkylation in DNA is a guanine residue (Hemminki and Hesso 1984, Koskinen *et al.*
15 2000b, Koskinen *et al.* 2000a, Latif *et al.* 1988, Savela *et al.* 1986). Styrene-7,8-oxide
16 forms adducts at the N7-, N²-, and O⁶-positions of guanine, the N1-, N3-, and N⁶-
17 positions of adenine, the N3-, N⁴-, and O²-positions of cytosine, and the N3-position of
18 thymine (Figure 5-3). *In vitro* studies indicated that N7- and N²-alkylguanine and O⁶-
19 adducts of guanine adducts were the most abundant, followed by adducts with
20 deoxycytidine (N3, N⁴, and O²), deoxyadenosine (N1, N3, and N⁶), and thymidine (N3)
21 (IARC 2002). The relative reactivity of the nucleosides with styrene-7,8-oxide are dG >
22 dC > dA > T, while the alkylation rates of guanine by styrene-7,8-oxide are
23 deoxyguanosine > single-stranded DNA > double-stranded DNA (Phillips and Farmer
24 1994, Savela *et al.* 1986). Vodička and Hemminki (1988) reacted radioactive styrene-7,8-
25 oxide with double- and single-stranded DNA. The N7-, N²-, and O⁶-guanine adducts
26 accounted for at least 95% of the total in single stranded DNA and formed in the
27 proportions 54:33:12. The proportions were 74:23:3.7 in double-stranded DNA,
28 indicating suppression at atoms that take part in hydrogen bonding in double-stranded
29 DNA (N² and O⁶). At neutral pH, styrene-7,8-oxide in solution with guanosine alkylated
30 the nucleoside mainly at the N7-position (57% of identified products), followed by the

1 N²- (28%) and O⁶-positions (15%) (Hemminki and Hesso 1984). When styrene-7,8-oxide
 2 was incubated *in vitro* with double-stranded DNA, the α and β forms of the N7-guanine
 3 adduct together constituted up to 74% of total adducts formed, while the α form of the
 4 N²-guanine adduct constituted about 3% and the O⁶-guanine adduct about 1% (Koskinen
 5 *et al.* 2001b, Vodicka *et al.* 2002a). The exact proportion of O⁶-guanine adducts has been
 6 difficult to determine because of their chemical instability; however, the half-life of the α
 7 isomer of O⁶-guanine adducts in double-stranded DNA has been estimated to be 1,320
 8 hours. Thymidine is a poor substrate for styrene-7,8-oxide, with only minor alkylation
 9 occurring at pH 7.4 and 37°C at the N3-position (Koskinen *et al.* 1999).

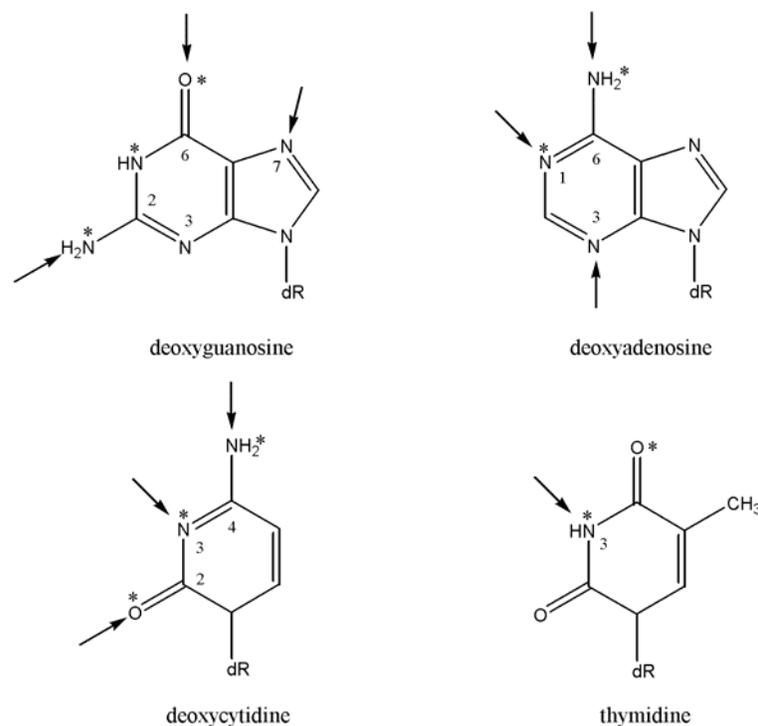


Figure 5-3. Styrene-7,8-oxide binding sites in DNA (from Vodicka *et al.* 2002a)
 Styrene-7,8-oxide-binding sites are indicated by arrows; base-pairing sites in DNA are labeled with asterisks.

10 A number of interconversions may occur with styrene-7,8-oxide nucleotide adducts. N1-
 11 adenine adducts can deaminate to form the corresponding hypoxanthine adduct, and N3-
 12 deoxycytidine adducts are rapidly deaminated to the corresponding deoxyuridine. N1-
 13 adenine adducts also may undergo rearrangement to the N⁶-adduct via the Dimroth

1 rearrangement, in which the adducted molecule moves from the ring nitrogen atom to the
2 exocyclic nitrogen atom (Barlow *et al.* 1998). O²-cytosine adducts also are unstable,
3 being prone to depyrimidation and interconversion between the α - and β -isomers
4 (Koskinen *et al.* 2000b). The chemically stable DNA adducts identified *in vitro* in the
5 highest proportions are α -N⁶-adenine, α -N²-guanine, and β -N3-uracil (Koskinen *et al.*
6 2001b); however, these adducts have not yet been identified *in vivo* in experimental
7 animals (Vodicka *et al.* 2006b). O⁶-guanine adducts account for about 1% of total
8 adducts (Vodicka *et al.* 1994). In the case of O⁶-guanine adducts, the α -isomer can
9 convert to the β -isomer in a base-catalyzed rearrangement (Moschel *et al.* 1986).

10 Structural data for both α - and β -N⁶-dA adducts of styrene-7,8-oxide in DNA is
11 available. The α -N⁶-dA adducts locate in the major groove, with their orientation being
12 dependent upon stereochemistry. Adducts with *R*-stereochemistry orient in the 5'
13 direction; whereas, those with *S*-stereochemistry orient in the 3' direction (Feng *et al.*
14 1995, 1996, Stone and Feng 1996). While the adducts with *S*-tereochemistry induce a
15 slight bend in the duplex, those with *R*-stereochemistry do not (Le *et al.* 2000). The
16 structures of the diastereomeric α -N⁶-dA adducts mispaired with dC, have also been
17 examined, both in the 5'-CXA-3' sequence and the 5'-AXG-3' sequence. This represents
18 the putative intermediate leading to A to G transitions. In the former sequence, the adduct
19 with *S*-stereochemistry remains in the major groove and oriented in the 3'-direction, as
20 observed for the corresponding adduct paired correctly with thymine. A shift of the
21 modified adenine toward the minor groove results in the styrenyl ring stacking with the
22 5'-neighboring cytosine, which shifts toward the major groove. A wobble A•C base pair
23 is not observed. In this mismatched duplex, the adduct of *R*-stereochemistry is disordered
24 (Painter *et al.* 1999). In the 5'-CXA-3' sequence, the thermodynamic stability of both the
25 mismatched *R*- and *S*-adducts is dependent upon pH. At neutral pH, both exhibit
26 significant structural perturbations and lower T_m values, as compared with the 5'-CXA-3'
27 sequence. This is attributed to reorientation about the adenine C6-N⁶ bond. For the adduct
28 of *R*-stereochemistry, the styrenyl moiety remains oriented in the major groove but now
29 orients in the 3'-direction. For the adduct with *S*-stereochemistry, the styrene ring inserted
30 into the duplex, approximately perpendicular to the helical axis of the DNA, but now in

1 the 5'-direction (Simeonov *et al.* 2000). The increased tether length of the β -styrene-7,8-
2 oxide N⁶-dA adducts results in two changes in structure as compared with the α -styrene
3 adducts. First, less distortion is introduced into the duplex. For both the *R*- and *S*- β -N⁶-
4 dA adducts, the styrenyl moiety is accommodated within the major groove of the duplex
5 with little steric hindrance. Second, it mutes the influence of stereochemistry, such that in
6 contrast to the α -N⁶-dA adducts, either the *R*- or *S*-stereoisomeric β -N⁶-dA adducts
7 exhibit similar conformations within the major groove (Hennard *et al.* 2001).

8 5.4.2 *In vitro* studies

9 This section reviews *in vitro* studies of DNA adduct formation (5.4.2.1), DNA damage
10 and repair (5.4.2.2), mutagenicity (5.4.2.3), and cytogenetic markers (5.4.2.4) for styrene
11 and styrene-7,8-oxide.

12 5.4.2.1 DNA adducts

13 The various types of DNA adducts associated with exposure to styrene or styrene-7,8-
14 oxide (based on the binding site on the nucleotide) are shown in Figure 5-3.

15 *Styrene*

16 No *in vitro* DNA adduct studies with styrene were identified.

17 *Styrene-7,8-oxide*

18 IARC (1994b, 2002) and Philips and Farmer (1994) reviewed several *in vitro* studies on
19 DNA adduct formation in nucleosides, calf thymus or fish testis DNA, and in DNA in
20 mammalian and human cells exposed to styrene-7,8-oxide. Studies in cellular systems are
21 summarized in Table 5-2. Exposure of 9L cells [rat brain gliosarcoma cells] to 1 mM
22 styrene-7,8-oxide, [a concentration that has resulted in increased chromosome aberrations
23 in human lymphocytes *in vitro*], for 24 hours resulted in formation of several DNA
24 adducts (Liu *et al.* 1988a). DNA adduct formation in human cells exposed to styrene-7,8-
25 oxide has been studied in cultured peripheral blood lymphocytes (PBLs), human
26 embryonic lung fibroblasts (HEL), and keratinocytes. DNA adducts (O⁶-guanine, N7-
27 guanine, or N²-guanine) were induced in all cell lines. These studies indicated a dose-
28 related increase in DNA adducts, persistence of the O⁶-guanine adducts, and a correlation
29 with single-strand breaks (see Section 5.4.2.2). In HEL cells, lower levels of N7-guanine

1 adducts were observed after 18-hour than 3-hour exposures; this finding could be due to
 2 the conversion of N7-guanine adducts into abasic sites either spontaneously or through
 3 the DNA repair process (Vodicka *et al.* 1996). Pauwels and Veulemans (1998) also
 4 reported N7-guanine adducts of styrene-7,8-oxide with human DNA when whole blood
 5 was incubated with styrene-7,8-oxide at 9.4 to 460 mM; however, they did not report the
 6 numbers of adducts formed.

7 Adduct persistence varies by binding site, and the time of exposure to the alkylating
 8 agent largely determines the relative proportions of the various DNA adducts. The
 9 available data indicate that the N7-adducts are lost, with an estimated half-life of about
 10 19 hours in one study (Vodicka *et al.* 1996). Some data indicate that O⁶ adducts are stable
 11 and can build up over time from chronic low-level exposures (Bastlová *et al.* 1995,
 12 Vodicka *et al.* 1999, Vodicka *et al.* 1994). An apparent saturation level is reached
 13 considerably faster for the N7-guanine and N3-adenine adducts, because these
 14 nucleotides depurinate more readily than adducts formed at positions involved in base-
 15 pairing (see Figure 5-3) (Vodicka *et al.* 2002a).

Table 5-2. Styrene-7,8-oxide DNA adducts formed in mammalian cells *in vitro*

Cell type	Treatment		Adducts		Reference
	Conc. (µM)	Duration	Type	No. per 10 ⁸ dNp	
Rat 9L (gliosarcoma cells)	1,000	24 h	NR	1,950	Liu <i>et al.</i> 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 <i>et al.</i> 1995
	200	6 d	O ⁶ -guanine	1.2	
	600	24 h	N7-guanine N ² -guanine	580 7	Vodicka <i>et al.</i> 2002a
Human embryonic lung fibroblasts	10 50 100	3 h	N7-guanine	10 ^a 20 ^a 35 ^a	Vodicka <i>et al.</i> 1996
	10 50 100	18 h	N7-guanine	4 ^a 8 ^a 9 ^a	
	100 300	NR	N7-guanine	140 470	
Human keratinocytes	100 300	NR	N7-guanine	140 470	Vodicka <i>et al.</i> 2002a

Cell type	Treatment		Adducts		Reference
	Conc. (μ M)	Duration	Type	No. per 10^8 dNp	
	100	NR	N ² -guanine	0.8	
	300			1.4	

dNp = dinucleotide pair; NR = not reported.

^a Levels were estimated from figures.

1 5.4.2.2 DNA damage and repair

2 Assays for DNA damage may detect double-strand and single-strand breaks, alkali-labile
3 sites, oxidative DNA base damage, or crosslinks (DNA-DNA or DNA-protein). In
4 addition, base and nucleotide excision repair processes also induce transient breaks;
5 therefore, a high level of breaks may indicate high levels of DNA damage or repair
6 (Collins *et al.* 1997). Collins *et al.* noted that single-strand breaks are quickly repaired
7 and are not generally regarded as significantly lethal or mutagenic lesions. Genotoxic
8 agents may directly induce single-strand breaks or may create apurinic/apyrimidinic sites,
9 which are converted to strand breaks in the alkaline electrophoresis solution used in the
10 comet assay.

11 Styrene

12 Two studies in *Escherichia coli* strain PQ37 gave somewhat conflicting results for DNA
13 repair as measured by the SOS chromotest. One study gave negative results, and the other
14 was inconclusive, showing positive results but no dose-response relationship (IARC
15 1994a)

16 Only one study of single-strand breaks was identified. Sina *et al.* (1983) developed an
17 alkaline elution/rat hepatocyte assay to measure DNA single-strand breaks and tested the
18 method on 91 compounds, including styrene and styrene-7,8-oxide. Rat hepatocytes were
19 treated with styrene at concentrations of 0.03, 0.3, and 3 mM for 3 hours. Single-strand
20 breaks were significantly increased at the highest concentration compared with controls.

21 Styrene-7,8-oxide

22 IARC (1994b) reviewed four studies of SOS induction in bacteria (one in *S. typhimurium*
23 and three in *E. coli*). Three studies resulted in a positive response without metabolic
24 activation. One study in *E. coli* was negative with or without metabolic activation.

1 Styrene-7,8-oxide induced single-strand breaks and DNA damage in rat hepatocytes
2 (Sina *et al.* 1983), neuroadrenergic (pheochromocytoma) rat PC-12 cells (Dydbukt *et al.*
3 1992), human PBLs and HEL cells (Bastlová *et al.* 1995, Laffon *et al.* 2001b, 2002b,
4 Laffon *et al.* 2003b, Vodicka *et al.* 1996), rat and human testicular cells (Bjørge *et al.*
5 1996), and Chinese hamster V79 lung fibroblast cells (Herrero *et al.* 1997). The results
6 are summarized in Table 5-3. These studies indicated that single-strand breaks increased
7 in a dose-related manner and were correlated with formation of DNA adducts.
8 Additionally, Bastlová *et al.* (1995) and Vodicka *et al.* (1996) showed that single-strand
9 breaks in DNA in human PBLs and HEL cells were repaired rapidly, with approximate
10 half-lives of 40 to 80 minutes. Vodicka *et al.* (1996) concluded that N7-guanine adducts
11 were important in the formation of single-strand breaks, because of their strong
12 correlation in HEL cells. Higher concentrations of styrene-7,8-oxide were required to
13 induce single-strand breaks in Chinese V79 hamster cells engineered to express human
14 mEH than in cells lacking this enzyme, suggesting that mEH might have protective
15 effects (Herrero *et al.* 1997) (see Sections 5.1.3.6 and 5.3.2 regarding the role of mEH in
16 detoxification). Marczynski *et al.* (1997b) exposed human whole blood to styrene-7,8-
17 oxide for 1.5 to 4 hours and reported that the observed degradation of high molecular
18 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1997b
	10–200 μM (0.5 h)	Comet assay	50 μM	+ DNA damage was correlated with SO concentration	Laffon <i>et al.</i> 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 <i>et al.</i> 2002b
	50–200 μM (0.5 h)	Comet assay	50 μM	+ Increased damage was associated with decreasing EH activity	Laffon <i>et al.</i> 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 <i>et al.</i> 1996
Human and rat testicular cells	10–300 μM (0.5 h)	Alkaline filter elution	100 μM	+	Bjørge <i>et al.</i> 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.

^aReported by Marczynski *et al.* (1997b) as dose in μmol .

1 5.4.2.3 Mutagenicity

2 The mutagenicity of styrene and styrene-7,8-oxide has been investigated in a number of
3 *in vitro* systems and is discussed below and summarized in Table 5-4.

4 Styrene

5 Most of the studies on styrene mutagenicity in bacterial systems were conducted two or
6 three decades ago, and the results were reviewed by IARC (1994a, 2002). Briefly, in
7 *Salmonella typhimurium* strains, the majority of studies on reverse mutation gave
8 negative results without metabolic activation. A few studies reported positive results with
9 metabolic activation in TA100, TA1530, and TA1535, which detect base-pair
10 substitutions. In eukaryotes, positive results were reported for *Saccharomyces cerevisiae*
11 (reverse mutation and gene conversion), *Drosophila melanogaster* (sex-linked recessive
12 mutation in one study), and *hprt* mutations in Chinese hamster V79 cells with metabolic
13 activation (one study). Negative results were reported in two studies of forward mutations
14 in *Schizosaccharomyces pombe*, one study of *w/w+* somatic mutations in *D.*
15 *melanogaster*, and two studies of *hprt* mutations in Chinese hamster V79 cells without
16 metabolic activation (IARC 1994a, 2002).

17 Styrene-7,8-oxide

18 Styrene-7,8-oxide was mutagenic in the majority of *in vitro* systems, primarily without
19 metabolic activation. Positive results were found in *S. typhimurium*, *E. coli* (SOS
20 chromotest), *Klebsiella pneumoniae*, *S. cerevisiae*, *S. pombe*, and *D. melanogaster*
21 (mixed results) and at the *tk* locus in mouse lymphocytes, the *hprt* locus in Chinese
22 hamster V79 cells, and the *HPRT* locus in human T lymphocytes and B lymphoblastoid
23 cells (weakly positive). Negative results were found in the *D. melanogaster w/w+*
24 somatic mutation assay (Rodriguez-Arnaiz 1998). Bastlová and Podlutsky (1996)
25 characterized *HRPT* mutations induced by styrene-7,8-oxide in T lymphocytes. They
26 found that the dominating base substitution in the *HPRT* gene was an A·T→G·C
27 transition, followed by G·C→T·A and A·T→T·A transversions. The DNA adducts
28 resulting in some of these base substitutions were tentatively identified as N⁶-
29 alkyladenine (A·T→G·C transition) and N7-alkylguanine (G·C→T·A and A·T→T·A
30 transversions).

1 Several studies also have compared the mutagenicity of styrene-7,8-oxide enantiomers
 2 (Pagano *et al.* 1982, Seiler 1990, Sinsheimer *et al.* 1993). Pagano *et al.* (1982)
 3 investigated the mutagenic properties of the *R*-enantiomer, the *S*-enantiomer, and a
 4 racemic mixture of *R*- and *S*-enantiomers in *S. typhimurium* TA100. The order of
 5 mutagenicity was *R*-enantiomer > racemic mixture > *S*-enantiomer. Seiler (1990)
 6 reported on similar studies with styrene-7,8-oxide enantiomers in *S. typhimurium* TA100;
 7 an intrinsic difference in the mutagenic activity of the enantiomers was strongly
 8 suggested by evidence of qualitative differences in their binding to DNA. Sinsheimer *et*
 9 *al.* (1993) also found the *R*-enantiomer to be a more potent mutagen in *S. typhimurium*
 10 than the *S*-enantiomer; however, these results were not predictive of *in vivo* genotoxicity
 11 in mice where the *S*-enantiomer rather than the *R*-enantiomer was associated with an
 12 increase in chromosomal aberrations and sister chromatid exchange (SCE).

Table 5-4. Mutagenicity of styrene and styrene-7,8-oxide *in vitro*

Test system	Styrene		Styrene-7,8-oxide	
	-S9	+S9	-S9	+S9
<i>S. typhimurium</i> (reverse mutation)	-	±	+	±
<i>E. coli</i> (SOS chromotest)	- ^a	NT	+	NT
<i>K. pneumoniae</i> (forward mutation)	NT	NT	+	NT
<i>S. cerevisiae</i> (reverse mutation & gene conversion)	+	NT	+ ^b	NT
<i>S. pombe</i> (forward mutation)	-	-	+	NT
<i>D. melanogaster</i> (sex-linked recessive)	+	NT	+	NT
<i>D. melanogaster</i> (somatic w/w+)	- ^c	NT	NT	NT
Chinese hamster V79 cells (<i>hprt</i>)	-	+	+	-
mouse lymphoma L5178Y (<i>tk</i>)	NT	NT	+	-
human lymphocytes (<i>HPRT</i>)	NT	NT	(+)	NT
human T lymphocytes (<i>HPRT</i>)	NT	NT	+	NT
human B lymphoblastoid cells (<i>HPRT</i>)	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).

^aNegative or inconclusive results in the SOS chromotest for DNA repair.

^bPositive results for gene conversion only.

^cA positive result was reported by IARC (2002) for insecticide-resistant strains, which have high
 bioactivation capacities.

1 5.4.2.4 Cytogenetic markers

2 The cytogenetic effects of styrene and styrene-7,8-oxide have been extensively reviewed
3 (Barale 1991, Cohen *et al.* 2002, IARC 1994a, 1994b, 2002, Scott and Preston 1994b)
4 and are summarized below. Both styrene and styrene-7,8-oxide cause cytogenetic damage
5 in various cell types tested *in vitro*. End points investigated include SCE, chromosomal
6 aberrations, micronuclei, and aneuploidy.

7 *Styrene*

8 Results of *in vitro* cytogenetic studies with styrene are summarized in Table 5-5. All
9 studies with human lymphocytes gave positive results. Scott and Preston (1994a) noted
10 that chromosomal aberrations and SCEs in human lymphocytes increased in the presence
11 of erythrocytes (i.e., in whole-blood cultures). Erythrocytes have the capacity to oxidize
12 styrene to styrene-7,8-oxide (see Section 5.1.3.5), while lymphocytes have the potential
13 to inactivate styrene-7,8-oxide through metabolism by mEH.

14 SCE were observed in rat and human lymphocytes and in Chinese hamster ovary (CHO)
15 cells under certain test conditions (IARC 1994a, 2002, Scott and Preston 1994a). In one
16 study (de Raat 1978), SCE were induced in CHO cells only when metabolic activation
17 (S9 fraction) was combined with incubation with cyclohexene oxide, an mEH inhibitor,
18 suggesting that styrene is metabolically activated to styrene-7,8-oxide but rapidly
19 inactivated by mEH. In another paper reporting six experiments with CHO cells (Norppa
20 and Tursi 1984), styrene at high concentrations (8 to 12 mM) caused SCE in one of three
21 experiments without metabolic activation and in two experiments in the presence of
22 human erythrocytes, but did not cause SCE in one experiment in the presence of S9. [The
23 high concentrations of styrene used in these experiments limit the interpretation of these
24 studies.]

25 Chromosomal aberrations were reported in studies with *Allium cepa* root-tip cells and
26 human lymphocytes exposed to styrene and in two of three studies in Chinese hamster
27 lung cells (weakly positive results). Micronucleus formation occurred in human
28 lymphocytes and *A. cepa* root-tip cells (IARC 1994a, 2002, Scott and Preston 1994a). A
29 strong c-mitotic effect and disordered anaphases were reported in *A. cepa* root-tip cells,

- 1 and aneuploidy occurred in human lymphocytes (Linnainmaa *et al.* 1978a, 1978b) but not
- 2 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 <i>et al.</i> 1983a
		–	0.7	+	Norppa <i>et al.</i> 1980a
		–	0.5	+	Norppa <i>et al.</i> 1983a
		–	1.0	+	Norppa and Vainio 1983
		–	0.01	+	Chakrabarti <i>et al.</i> 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 <i>et al.</i> 1983b
Chromosomal aberrations	Human lymphocytes (isolated cultures or whole blood) ^c	–	1.0	+	Jantunen <i>et al.</i> 1986
		–	2.6	+	Linnainmaa <i>et al.</i> 1978a, 1978b
		–	0.5	+	Pohlova and Sram 1985
		–	2.0	+	Jantunen <i>et al.</i> 1986
	Chinese hamster lung cells	–	2.4	–	Matsuoka <i>et al.</i> 1979
S9		2.4	(+)	Matsuoka <i>et al.</i> 1979	
–		1.0	(+)	Ishidate and Yoshikawa 1980	
	<i>A. cepa</i>	–	0.87	+	Linnainmaa <i>et al.</i> 1978a, 1978b
Micronuclei	Human lymphocytes (whole blood)	–	2.6	+	Linnainmaa <i>et al.</i> 1978a, 1978b
	<i>A. cepa</i>	–	1.7	+	Linnainmaa <i>et al.</i> 1978a, 1978b
Aneuploidy	Human lymphocytes (whole blood)	–	2.6	+	Linnainmaa <i>et al.</i> 1978b
	<i>D. melanogaster</i>	–	5	–	Penttila <i>et al.</i> 1980
C-mitosis	<i>A. cepa</i>	–	0.87	+	Linnainmaa <i>et al.</i> 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).

1 *Styrene-7,8-oxide*

2 Styrene-7,8-oxide induced cytogenetic effects at lower concentrations than did styrene,
3 and metabolic activation was not necessary. Results are summarized in Table 5-6. SCEs
4 occurred in human lymphocytes, CHO cells, and Chinese hamster V79 cells.
5 Chromosomal aberrations occurred in human lymphocytes and Chinese hamster V79
6 cells but not in *A. cepa*. Micronuclei were induced in human lymphocytes, Chinese
7 hamster V79 cells, and *A. cepa*. Linnainmaa *et al.* (1978a, 1978b) also reported anaphase
8 bridges in *A. cepa* cells, which induced micronuclei in successive telophases and
9 interphases. It was not possible to assess incidences of aneuploidy in human lymphocytes
10 exposed to styrene-7,8-oxide because of severe chromosome destruction.

11 *5.4.3 In vivo studies in experimental animals*

12 *5.4.3.1 DNA adducts*

13 As mentioned above, styrene does not bind to DNA unless metabolically activated to
14 styrene-7,8-oxide. The potential of styrene or styrene-7,8-oxide exposure to induce DNA
15 adducts in experimental animals was studied earlier through the use of radiolabeled
16 compounds, as reviewed by Phillips and Farmer (1994) and Cohen *et al.* (2002). These
17 studies generally showed low levels of DNA adducts in rats and mice following exposure
18 to styrene or styrene-7,8-oxide by various routes of administration. However, the reported
19 levels of DNA binding varied by factors of 20 to 50 among studies, for reasons that were
20 not completely understood. According to Phillips and Farmer, differences in route of
21 administration, methods of measurement, and losses from depurination should be
22 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	+	Norppa <i>et al.</i> 1980a
		0.15	+	Norppa <i>et al.</i> 1983a
		0.008	+	Pohlova and Sram 1985
		0.1	+	Zhang <i>et al.</i> 1993
		0.05	+	Lee and Norppa 1995
		0.05	+	Uuskula <i>et al.</i> 1995
		0.1	+	Chakrabarti <i>et al.</i> 1997
		0.05	+	Ollikainen <i>et al.</i> 1998
	CHO cells	0.18	+	Laffon <i>et al.</i> 2001b
	Chinese hamster V79 cells	0.17	+	de Raat 1978
0.12		+	Nishi <i>et al.</i> 1984 von der Hude <i>et al.</i> 1991	
Chromosomal aberrations	Human lymphocytes	0.59	+	Linnainmaa <i>et al.</i> 1978a, 1978b
		0.1	+	Fabry <i>et al.</i> 1978
		0.2	+	Norppa <i>et al.</i> 1981b
		0.024	+	Pohlova and Sram 1985
	Chinese hamster V79 cells	0.75	+	Turchi <i>et al.</i> 1981
	<i>A. cepa</i>	3.7	-	Linnainmaa <i>et al.</i> 1978a, 1978b
Micronuclei	Human lymphocytes	0.59	+	Linnainmaa <i>et al.</i> 1978a, 1978b
		0.1	+	Laffon <i>et al.</i> 2001b
	Chinese hamster V79 cells	0.75	+	Turchi <i>et al.</i> 1981
	<i>A. cepa</i>	3.7	+	Linnainmaa <i>et al.</i> 1978a, 1978b
Anaphase bridges	<i>A. cepa</i>	0.74	+	Linnainmaa <i>et al.</i> 1978a, 1978b

Source: adapted from Scott and Preston 1994a; IARC 1994a, 1994b, 2002.

+ = positive response; - = negative response.

^aLowest effective concentration or highest ineffective concentration.

1 Styrene

2 Adducts resulting from exposure to tritiated styrene were detectable in 2 of 4 lung
3 samples from female rats and in mouse liver but not in rat liver; however, no lung tissue
4 was collected from mice in this study (Cantoreggi and Lutz 1993). The earlier study by
5 Byfält-Nordqvist *et al.* (1985) with ¹⁴C-labeled styrene in NMRI mice reported adduct
6 values 20 to 50 times those reported by Cantoreggi and Lutz. Philips and Farmer (1994)
7 were not able to identify a reason for the difference although they noted that the methods
8 differed for route of administration (inhalation, ingestion, and i.p. injection) and in the

1 quantitation of radioactivity (coelution with adduct standards vs. measurement of total
2 radioactivity, but they did not consider these differences sufficient to explain the widely
3 differing results.

4 More recent studies have focused on the quantitative and qualitative determination of
5 specific styrene-induced DNA adducts (Boogaard *et al.* 2000b, Gamer *et al.* 2004,
6 Otteneder *et al.* 2002, Pauwels *et al.* 1996, Vodicka *et al.* 2001b, Vodicka *et al.* 2006a).
7 The studies are reviewed below and summarized in Table 5-7.

8 DNA adducts resulting from exposure to styrene were detected in tissues from male
9 NMRI mice in several studies by Vodicka and co-workers (Pauwels *et al.* 1996, Vodicka
10 *et al.* 2001b, Vodicka *et al.* 2006a). In the only study using i.p. injection (Pauwels *et al.*
11 1996), styrene was administered at 0 to 4.35 mmol/kg b.w., and tissues were collected 3
12 hours later. N7- and O⁶-guanine adducts were present in the lungs, liver, and spleen, but
13 N7 adducts were more abundant in all three tissues, and the lungs contained
14 approximately 30% more of these adducts than did the liver or spleen. The authors
15 pointed out that the liver would be expected to be exposed to styrene as the first-pass
16 organ, but they suggested that the balance between formation and detoxification of
17 styrene-7,8-oxide in the organs could explain the higher adduct levels in lung. DNA
18 adduct levels correlated with exposure level and formation of hemoglobin adducts.

19 In the inhalation studies, β-N7-guanine adducts were detected in the lungs but not liver,
20 and β-N1-adenine adducts were detected in both lungs and liver of male NMRI mice
21 exposed to styrene at 750 or 1,500 mg/m³ [175 or 350 ppm] 6 hours per day, 7 days per
22 week, for 1, 3, 7, or 21 days (Vodicka *et al.* 2001b, 2006a). Levels of both types of DNA
23 adducts in the lungs correlated significantly with styrene concentrations in blood as a
24 measure of styrene exposure. Levels of N7-guanine adducts were compared between the
25 lungs and the urine (with correction for depurination); the total N7-guanine adducts (23.0
26 adducts/10⁸ nucleotides) in the lungs of mice in the highest exposure group (1,500 mg/m³
27 [350 ppm] for 21 days) accounted for approximately 0.5% of the total N7-guanine
28 adducts measured by cumulative urinary excretion.

1 DNA adducts also were detected in liver, lungs, and isolated lung cells of male CD-1
2 mice and male Sprague-Dawley rats exposed to [ring-U-¹⁴C]styrene at 160 ppm by nose-
3 only inhalation for 6 hours (Boogaard *et al.* 2000b). Tissues were collected either
4 immediately or 42 hours after exposure. Low levels of N7-guanine adducts were detected
5 in both liver and lung; however, unidentified adducts [authors' term] were present in liver
6 at higher levels than the N7-guanine adducts. The level of N7 adducts and of two of the
7 three unidentified adducts increased from 0 to 42 hours. N7 adducts were the major
8 adduct type in the lungs, at a level of about 1 per 10⁸ nucleotides immediately after
9 exposure and at about half this level 42 hours after exposure. Adducts also were
10 measured in Clara cells and non-Clara cells. Adducts were analyzed in lung tissue from 2
11 mice and lung cells from 24 mice. N7-guanine adduct levels were similar in Clara cells,
12 non-Clara cells, and whole lung. After 42 hours, an unidentified adduct was detected at
13 levels of 6 per 10⁸ nucleotides in Clara cells and at 80 per 10⁸ nucleotides in non-Clara
14 cells. The authors stated that because of the small amount of DNA isolated from non-
15 Clara cells, this value had a large relative error (approximately 30%). The authors used
16 styrene metabolite standards to identify this adduct (which was the same for both lung-
17 cell types) and found that benzoic acid co-eluted with the compound. They speculated
18 that the most likely source was benzaldehyde, which is a putative intermediate in the
19 metabolism of styrene from mandelic acid to hippuric acid; however, they also suggested
20 that this adduct could be an artifact resulting from the radioactive styrene used for the
21 exposure.

22 N7-guanine adducts were detected in both rat liver and lung; however, the unidentified
23 adducts that were present in mouse liver at higher levels than the N7-guanine adducts
24 (see above) were not detected in rat liver (Boogaard *et al.* 2000b). One rat was used for
25 the adduct analysis from lung tissue and five rats were used for the cell-type analysis. N7-
26 adduct levels were approximately 1 adduct per 10⁸ nucleotides immediately after
27 exposure, and about half this level at 42 hours after exposure. Type II cells isolated from
28 lungs of styrene-exposed rats contained higher levels of N7-guanine adducts (2 adducts
29 per 10⁸ nucleotides) than whole lung.

1 Otteneder *et al.* (2002) did not detect O⁶-guanine adducts in the lungs or liver of CD-1
 2 mice exposed to styrene at 40 or 160 ppm for 2 weeks. Gamer *et al.* (2004) also reported
 3 that no changes were found in 8-hydroxy-deoxyguanosine as an indicator of oxidative
 4 stress after either a single 6-hour exposure or multiple exposures of female CD-1 mice to
 5 styrene by inhalation; however, they did find that glutathione was depleted in lung
 6 homogenates.

7 In CD-1 rats exposed for 2 years to styrene at 1,000 ppm (the highest level tested), both
 8 α - and β -O⁶-guanine adducts were detectable in the liver at levels of 90 per 10⁸
 9 nucleotides in males and 80 per 10⁸ nucleotides in females (Otteneder *et al.* 2002). An
 10 isomer-enriched analysis was used for the 1,000-ppm samples only, and all of the lung
 11 tissue was used for histopathological analysis. However, no O⁶-guanine adducts were
 12 detected in the lungs or liver of CD-1 rats exposed to styrene at 500 ppm for 2 weeks.

13 Overall, the N7- and O⁶-guanine adducts were found most often in these studies. As
 14 noted in Section 5.4, the N7-guanine adducts are the most common form resulting from
 15 exposure to styrene, but the O⁶-guanine adducts are more persistent, which may explain
 16 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 <i>et al.</i> 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	β N7-guanine	lung: 23 liver: ND	Vodicka <i>et al.</i> 2001b
		β N1-adenine	lung: 0.6 liver: 0.2	Vodicka <i>et al.</i> 2006a

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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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.

1 Styrene-7,8-oxide

- 2 Philips and Farmer (1994) reported that very low levels of DNA adducts were formed in
3 the forestomach [the target tissue for styrene-7,8-oxide–induced tumors] and liver when

1 tritiated styrene-7,8-oxide was administered by gavage to rats and by i.p. injection to
2 mice (Cantoreggi and Lutz 1992, Lutz *et al.* 1993). An earlier report by Byfält-Nordqvist
3 *et al.* (1985) in which tritiated styrene-7,8-oxide or styrene was administered by i.p.
4 injection to NMRI mice reported that alkylation of DNA in liver, brain, and lung
5 exceeded that in spleen and testis, but the forestomach was not examined.

6 5.4.3.2 DNA damage and repair

7 Results from studies of DNA damage in experimental animals exposed to styrene or
8 styrene-7,8-oxide are summarized in Table 5-8 and discussed below. Most of the studies
9 used the alkaline single-cell gel electrophoresis (comet) assay. The comet assay involves
10 embedding single cells in agarose gel followed by lysis in alkali and electrophoresis
11 (Vaghef and Hellman 1998). This assay can detect strand breaks, alkali-labile sites
12 (converted to single-strand breaks under the alkaline conditions of the assay), oxidative
13 base damage, crosslinks, and DNA repair.

14 Styrene

15 DNA damage was observed in two studies in mice exposed to styrene by i.p. injection
16 (Vaghef and Hellman 1998, Walles and Orsen 1983). Walles and Orsen (1983)
17 administered styrene at 1.7 to 10.1 mmol/kg b.w. [177 to 1,052 mg/kg b.w.] to male
18 NMRI mice and determined DNA damage in kidney, liver, lung, testis, and brain at 1 to
19 24 hours after injection. DNA damage was increased in all tissues examined at 1 hour,
20 and the levels were still elevated at 24 hours in all tissues but the liver. Vaghef and
21 Hellman (1998) determined DNA damage in peripheral blood lymphocytes, bone
22 marrow, liver, and kidney cells at 4 and 16 hours after i.p. injection of 100 to 500 mg/kg
23 b.w. of styrene to female C57BL/6 mice. Significant increases in DNA damage were
24 found in all tissues at both 4 and 16 hours.

25 Inhalation studies are usually conducted over a long period, to ensure that equilibrium
26 between DNA damage and repair is reached; however, only subacute inhalation studies
27 of styrene have been conducted. Vodicka *et al.* (2001b) exposed male NMRI mice to
28 styrene by inhalation at a concentration of 750 or 1,500 mg/m³ [175 or 350 ppm] for 7 or
29 21 days; they found significant increases in DNA damage only in lymphocytes at 7 days,
30 and not in bone marrow or liver cells. Endonuclease III-sensitive sites in bone marrow

1 were increased significantly at 21 days at both exposure levels, suggesting an increase in
2 accumulation of abasic sites.

3 Only one study that examined DNA damage in styrene-exposed rats was identified.
4 Kligerman *et al.* (1993) exposed female F344 rats to styrene by inhalation at 125, 250, or
5 500 ppm, 6 hours per day for 14 consecutive days. No significant increase in DNA
6 damage was detected.

7 Clay (2004) reported that styrene induced DNA damage and repair in female CD-1 mice
8 in an assay for unscheduled DNA synthesis (UDS). Groups of 6 mice were exposed to
9 styrene by inhalation at either 125 or 250 ppm for 6 hours. A positive control group was
10 administered *N*-nitrosodimethylamine (NDMA) at 10 mg/kg b.w. by gavage. Groups of 3
11 mice were killed for tissue collection at 2 and 16 hours after exposure to styrene or
12 NDMA. No increase in UDS was observed for any of the animals exposed to styrene, but
13 the positive control induced increases in UDS that the author characterized as
14 appropriate.

15 *Styrene-7,8-oxide*

16 After a single i.p. injection of styrene-7,8-oxide, single-strand breaks or alkali-labile sites
17 in DNA were increased in male NMRI mice (in kidney, liver, lung, testis, and brain)
18 (Walles and Orsen 1983), male CD-1 mice (in liver, lung, kidney, spleen, and bone
19 marrow) (Sasaki *et al.* 1997), female C57BL/6 mice (in liver, lymphocytes, bone marrow,
20 and kidney) (Vaghef and Hellman 1998), and male ddY mice (in stomach, colon, liver,
21 kidney, urinary bladder, lung, brain, and bone marrow) (Tsuda *et al.* 2000). Tsuda *et al.*
22 (2000) and Sasaki *et al.* (1997) took measurements at multiple time points (3 to 24 hours)
23 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 <i>et al.</i> 2001b
Female Fischer rats (PBLs)	125–500 ppm, 6 h/d, for 2 wk (inhalation)	comet assay	–	Kligerman <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 2000

+ = positive; ± = equivocal – = negative.

1 **5.4.3.3 Mutations**

2 No studies evaluating specific gene mutations in experimental animals exposed to styrene
3 or styrene-7,8-oxide were identified.

4 **5.4.3.4 Cytogenetic studies**

5 Cytogenetic effects include SCE, chromosomal aberrations, micronuclei, aneuploidy, and
6 polyploidy. *In vivo* cytogenetic studies of styrene and styrene-7,8-oxide exposure have
7 been conducted in mice, rats, and hamsters and are reviewed below. Cohen *et al.* (2002)
8 reviewed the cytogenetic effects of styrene and styrene-7,8-oxide in experimental animals

1 and reported that positive effects were seen only at high exposure levels that are not
2 likely relevant for human exposure; however, because human exposures are usually of
3 much longer duration, the authors suggested that lower exposure levels over longer
4 exposure periods could have clastogenic effects in animals.

5 *Styrene*

6 IARC (1994a) and Scott and Preston (1994a) reviewed three to eight studies each for
7 SCE, chromosomal aberrations, and micronuclei in experimental animals (rats and mice)
8 exposed to styrene by inhalation, i.p. injection, or gavage. All of the studies gave positive
9 or weakly positive results for SCE; SCE were detected in liver, alveolar macrophages,
10 lungs, bone marrow, splenocytes (weakly positive), and lymphocytes of mice and
11 splenocytes and lymphocytes (weakly positive) of rats. In contrast, all the studies except
12 one (for each end point) gave negative results for chromosomal aberrations and
13 micronuclei. Polyploidy was observed in Wistar rat bone-marrow cells following
14 administration of styrene by inhalation at 300 ppm for 11 weeks. Most studies were of
15 short duration (≤ 2 weeks). One inhalation study lasted for 12 months but did not report
16 increased incidences of chromosomal aberrations in rat bone marrow following exposure
17 to concentrations up to 1,000 ppm. Results from the studies reviewed by IARC (1994a,
18 2002) and Scott and Preston (1994a) are summarized in Table 5-9.

19 Only a few studies were identified that examined cytogenetic effects in experimental
20 animals exposed to styrene and were published after the IARC (1994a) review. IARC
21 (2002) reviewed one additional study of SCE and chromosomal aberrations in F344 rats
22 exposed to styrene at $4,260 \text{ mg/m}^3$ [1,000 ppm] for 4 weeks. The results were negative.
23 The genotoxicity of styrene and 1,3-butadiene was evaluated in B6C3F₁ mice exposed for
24 8 hours by inhalation (Leavens *et al.* 1997). Butadiene-exposed mice exhibited increased
25 micronuclei in bone marrow, while styrene-exposed mice did not. In another study, male
26 NMRI mice exposed to styrene at $1,500 \text{ mg/m}^3$ [350 ppm] had significantly increased
27 micronuclei in bone marrow after 7 days of exposure but not after 21 days of exposure
28 (Vodicka *et al.* 2001b). However, when this study was repeated, there was no evidence of
29 clastogenicity (micronuclei or chromosomal aberrations) in male NMRI mice exposed to
30 styrene at 750 or $1,500 \text{ mg/m}^3$ [175 or 350 ppm] for 1, 3, 7, 14, or 21 days [micronuclei

1 were evaluated independently by two laboratories] (Engelhardt *et al.* 2003). The authors
2 suggested that the positive result in the first experiment might have been the result of
3 some unidentified experimental variation, because the results were inconsistent between
4 time points.

5 *Styrene-7,8-oxide*

6 Fewer studies have examined the cytogenetic toxicity of styrene-7,8-oxide (IARC 1994b,
7 Scott and Preston 1994a). Results are summarized in Table 5-9. IARC (2002) did not
8 review any additional studies of clastogenic effects in experimental animals exposed to
9 styrene-7,8-oxide. SCEs were not increased in Chinese hamster bone-marrow cells
10 following inhalation or i.p. injection of styrene-7,8-oxide or in mouse bone-marrow cells
11 following inhalation exposure. Positive results for SCE were found in mouse bone-
12 marrow cells following a single i.p. injection of 100 mg/kg b.w., and weakly positive
13 results in mouse liver cells and alveolar macrophages following inhalation exposure.
14 Chromosomal aberrations were increased in mouse bone-marrow cells in two of three
15 studies, but not in Chinese hamster bone-marrow cells. Only two studies were available
16 that examined micronuclei in rodents exposed to styrene-7,8-oxide. In both studies, one
17 in BALB/c mice and the other in Chinese hamsters, micronucleus formation was not
18 increased in bone-marrow cells following a single i.p. injection of styrene-7,8-oxide at
19 250 mg/kg b.w.

20 One of these studies, Sinsheimer *et al.* (1993), investigated the effects of both the *R*- and
21 *S*-styrene-7,8-oxide isomers when administered by i.p. injection at 100 mg/kg b.w. to
22 male CD-1 mice. No effect on chromosomal aberrations per cell was reported with either
23 isomer, but the percentage of cells with SCE increased significantly ($P < 0.01$) following
24 exposure to the *S*-isomer (2.75 ± 0.50 , mean \pm SD) but not the *R*-isomer (1.75 ± 0.96),
25 compared with dimethylsulfoxide (DMSO) solvent controls (1.00 ± 0.82). The number of
26 SCE per cell was also significantly higher for the *S*-isomer than for the *R*-isomer or
27 DMSO. The mitotic index was significantly lower for both the *R*-isomer (2.74 ± 0.28)
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.

^aLED = lowest effective dose in positive studies; HID = highest ineffective dose in negative studies; LED given for studies with mixed results.

^bOne study was positive for the *S*-isomer but not the *R*-isomer.

^cPositive in 2 of 3 studies.

1 5.4.4 Studies in styrene-exposed workers

2 Many studies have examined the genetic effects of styrene in human populations;
3 however, interpretation of these studies is complicated by a number of factors that
4 increase the likelihood of both false positive and false negative results. These include less
5 control over study design details than in *in vitro* studies or animal bioassays, lack of
6 appropriate exposure data, and the need to control for possible confounding factors, such
7 as smoking or co-exposure to other chemicals used in the plastics industry (e.g., organic
8 peroxides, dichloromethane, hydroquinone, dimethylaniline, and maleic anhydride).

1 Other important study limitations include relatively small control groups, low sensitivity,
2 and high interindividual variability. [These facts have an impact on any human
3 biomonitoring study and, together with interlaboratory differences, may be responsible
4 for much of the ambiguity and inconsistency apparent in styrene population studies.]

5 Exposure to other chemicals that may also cause genetic damage is often correlated with
6 exposure to styrene. The following factors were considered by Cohen *et al.* (2002) to
7 increase the probability that an observed relationship is causal: (1) adequate statistical
8 control of confounders, (2) a positive dose-response relationship among exposed subjects,
9 and (3) a positive association across studies between a central measure of exposure and
10 the average magnitude of the increased frequency of the effect in each study. Results of
11 studies in styrene-exposed workers are summarized below for DNA adducts, DNA
12 damage, mutations, and cytogenetic markers.

13 *5.4.4.1 DNA adducts*

14 Results from studies in Bohemia (Czech Republic), the United States, and Germany are
15 summarized in Table 5-10. Very few studies were available on the detection of styrene-
16 specific DNA adducts in humans before 1994 (IARC 1994a). Liu *et al.* (1988b) reported
17 unidentified adducts in 1 styrene-exposed worker, and Vodicka *et al.* (1993) and Vodicka
18 and Hemminki (1993) reported O⁶-guanine adducts in lamination workers.

19 The two reports of DNA adducts in lamination workers by Vodicka and coworkers were
20 part of a series of studies (Koskinen *et al.* 2000a, 1995, Vodicka and Hemminki 1993,
21 1999, Vodicka *et al.* 1994) using samples collected from workers at a group of factories
22 in the same geographic area of Bohemia. [In many cases, the same individuals were
23 sampled repeatedly, and although the same individuals could be identified across some of
24 the studies, this was not possible in all cases.] Up to six samples were collected from each
25 individual between December 1992 and March 1995 (Vodicka *et al.* 1999). These six
26 occasions were (I) in December 1992, (II) in July 1993 one day before summer vacation,
27 (III) in August 1993 on the first day of work after two weeks of vacation, (IV) in
28 September 1993 after an additional month of work, (V) in February 1994, and (VI) in
29 March 1995. Data from these samplings are reported in Table 5-10. Two groups of
30 controls were used in this study: 7 factory controls and 8 laboratory controls (increased to

1 13 for sampling VI). The factory controls were sampled on occasions II through V, and
2 the laboratory controls were sampled on occasions V (N = 8) and VI (N = 13);
3 sampling VI was reported in the study by Vodicka *et al.* (1999) and included data for
4 laboratory controls only. These studies also included measurements of single-strand
5 breaks in DNA (see Section 5.4.4.2) and *HPRT* mutations in the same groups of workers
6 (see Section 5.4.4.3).

7 The results for O⁶-guanine adducts from sampling I were reported in Vodicka *et al.*
8 (1993) for 23 hand-lamination workers. The workers were divided into 2 groups that
9 differed by styrene exposure duration and levels, and adduct levels did not differ between
10 the controls and either group. The results for samplings II, III, and IV for samples from 7
11 or 9 workers (see Vodicka *et al.* 1999) were reported as part of a study of the persistence
12 of O⁶-guanine adducts (Vodicka *et al.* 1994). Vodicka *et al.* (1995) also reported the
13 results for O⁶-guanine adducts from samplings II, III, and IV and added results for
14 sampling V. [In the Vodicka *et al.* (1995) publication, the December 1992 results
15 reported by Vodicka *et al.* (1993) were not included, and the other samplings were
16 numbered I through IV.] Levels of styrene-specific DNA adducts were significantly
17 higher in workers than in controls at all sampling times before and after vacations for
18 samplings II, III, and IV, but there was no significant difference between samplings for
19 the exposed workers. Vodicka *et al.* (1994) therefore concluded that removal of specific
20 O⁶-styrene adducts from DNA was very slow. The results of the final sampling (VI) from
21 this group of workers was reported by Vodicka *et al.* (1999) together with occupational
22 exposure data from the earlier samplings. Separate values were reported for 11 workers
23 and 10 controls (of a total of 13 sampled in each group) and for the 8 workers and an
24 unspecified number of controls studied in previous samplings.

25 In the series of six consecutive samplings over 3 years described above, no tendency of
26 O⁶-guanine adducts to accumulate was reported, suggesting a well-established
27 equilibrium between DNA adduct formation and removal in chronically and highly
28 exposed hand-lamination workers (Vodicka *et al.* 1999). Although this study did not find
29 continued accumulation of O⁶-guanine adducts, Vodicka *et al.* (1994) interpreted the

1 relatively constant levels of these adducts over time, including time away from work for
2 vacations, as evidence for their persistence.

3 In addition to the O⁶-guanine adducts reported in the studies summarized above, other
4 types of adducts (e.g., β-N1-adenine, N²-guanine, and 8-hydroxy-2'-deoxyguanosine)
5 have been measured in styrene-exposed workers (Horvath *et al.* 1994, Koskinen *et al.*
6 2001a, Marczynski *et al.* 1997a, Rappaport *et al.* 1996, Vodicka *et al.* 2003). In studies in
7 Bohemia, low levels of β-N1-adenine adducts were detected in styrene hand-lamination
8 workers by a high-performance liquid chromatography–based method, but the adduct
9 levels were not significantly higher in workers than in controls (Koskinen *et al.* 2001a,
10 Vodicka *et al.* 2003). In studies of U.S. workers, N²-guanine adducts and a second
11 unidentified adduct were detected in 48 workers of both sexes employed in a boat-
12 manufacturing facility where mean styrene exposure was 64 mg/m³ [15 ppm] (range = 1
13 to 235 mg/m³ [0.2 to 55 ppm]) (Horvath *et al.* 1994, Rappaport *et al.* 1996). [However,
14 these studies included no controls.] Marczynski *et al.* (1997a) reported on 17 styrene-
15 exposed boat builders in Germany (aged 23 to 60) and 67 age-matched healthy volunteers
16 without prior exposure to styrene. Levels of 8-hydroxy-2'-deoxyguanosine adducts [an
17 indicator of oxidatively damaged DNA] were significantly higher in the workers than in
18 the controls.

19 Levels of β-N1-adenine adducts were significantly correlated with measures of styrene
20 exposure, including styrene in exhaled air ($r = 0.613$, $P = 0.007$), styrene in blood ($r =$
21 0.558 , $P = 0.003$), and urinary mandelic acid ($r = 0.836$, $P = 0.0003$) (Koskinen *et al.*
22 2001a), and styrene at the workplace ($r = 0.730$, $P < 0.001$), styrene in blood ($r = 0.605$,
23 $P < 0.001$), and urinary mandelic acid ($r = 0.670$, $P = 0.001$) (Vodicka *et al.* 2003).
24 Significant correlations between adducts and styrene exposure were also reported for the
25 population studied by Horvath *et al.* (1994) and Rappaport *et al.* (1996) (for styrene in
26 workplace air and N²-guanine adducts, $r = 0.244$, $P = 0.049$; for unidentified adducts, $r =$
27 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 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 <i>et al.</i> 1999
9/11	7.8	[18]	106	β-N1-adenine	0.08 ± 0.01	≤ 0.04 ^f	Koskinen <i>et al.</i> 2001a
19/7	14.1	[40]	NR	β-N1-adenine	0.3 ± 0.05	≤ 0.04 ^f	Vodicka <i>et al.</i> 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 <i>et al.</i> 1994 Rappaport <i>et al.</i> 1996
Germany							
17/67	1–> 10	NR	NR	8-OH-2α-deoxyguanosine	2,230 ± 540***	1,520 ± 450	Marczynski <i>et al.</i> 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/m³ 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 \pm SE.

1 5.4.4.2 DNA damage and repair

2 Pero *et al.* (1982) tested the sensitivity of human lymphocytes to stimulation of UDS by
3 *N*-acetoxy-2-acetylaminofluorene (NA-AAF) after exposure to styrene *in vivo* or *in vitro*.
4 UDS resulting from exposure to NA-AAF *in vitro* was significantly greater in
5 lymphocytes obtained from workers in a Swedish fiberglass-reinforced polyester plastic
6 factory exposed to styrene at 1 to 40 ppm than in lymphocytes from workers in a
7 mechanical industry in the same town. In lymphocyte cultures exposed to styrene at 0 to
8 750 μ M, NA-AAF-induced UDS was increased significantly ($P < 0.001$) compared with
9 the mean level of unexposed controls, and there was a significant ($P < 0.001$) linear
10 correlation with styrene concentration up to 100 μ M, above which the effect remained
11 elevated. The authors concluded that styrene could make lymphocytes more sensitive to
12 other genotoxic exposures and suggested that one potential mechanism could be
13 induction of mixed-function oxygenase activity by styrene, leading to increased
14 metabolism and activation of genotoxins such as NA-AAF.

15 DNA repair capacity was measured in lymphocytes from 14 styrene-exposed boat
16 builders and 7 controls from the wood manufacturing industry in an X-ray challenge
17 assay (Oberheitmann *et al.* 2001). Lymphocytes obtained were exposed to X-rays, and
18 the rate of exchange-type chromosomal aberrations per 100 metaphases was determined.
19 However, the duration of radiation exposure was different for the exposed and control
20 cultures, so the results could not be used for comparison. The authors compared the
21 results with those for 2 historical control subjects, who were individuals from the
22 research institute (the authors noted that the comparison could only be exploratory).
23 Significantly more chromosomal aberrations were found in the lymphocytes from
24 styrene-exposed workers than in the historical controls. In the exposed group, the
25 challenge response was significantly correlated with cumulative lifetime exposure to
26 styrene (years of exposure), but not with the current exposure (measured as styrene in the
27 blood). The authors concluded that their results were consistent with the hypothesis that
28 long-term exposure to styrene affects DNA repair activities in humans.

29 A significant positive correlation was observed between exposure parameters and rates of
30 base-excision repair (irradiation-specific repair and the repair of oxidatively damaged

1 DNA) (Vodicka *et al.* 2004a). Peripheral lymphocytes from styrene-exposed workers at
2 three plants or from controls working in a regional hygienic station were compared for
3 their ability to repair single-strand breaks induced by γ -rays *in vitro*. Repair rates
4 (reported as SSB/ 10^9 Da) were significantly higher for Plant A (mean \pm SD = $0.94 \pm$
5 0.32 , $P = 0.023$), Plant B (0.96 ± 0.44 , $P = 0.016$), and Plant C (1.63 ± 0.41 , $P = 0.001$)
6 than for controls (0.55 ± 0.64). Across the three plants, the rate of DNA repair correlated
7 significantly ($r = 0.308$, $P = 0.031$) with styrene concentration in the blood. DNA repair
8 increased with increasing styrene air concentration, but differed significantly from the
9 controls only for the high-exposure group (exposed to styrene at $> 50 \text{ mg/m}^3$ [12 ppm]; P
10 = 0.034). The authors suggested that particular DNA repair pathways might be induced
11 by styrene exposure.

12 Slyskova *et al.* (2007) compared the capacity to repair oxidatively damaged DNA in
13 mononuclear leukocytes obtained from 24 lamination workers occupationally exposed to
14 styrene for an average of 14.6 years and 15 unexposed controls. The DNA-repair capacity
15 was moderately higher in the exposed group compared with the controls, but the
16 difference was not significant. There was no significant correlation between the DNA-
17 repair capacity and styrene exposure or biomarkers of genotoxic effects (strand breaks,
18 DNA adducts, chromosomal aberrations, or *HPRT* mutant frequencies). The authors
19 suggested that the lack of a significant difference was most likely related to inter-
20 individual variability in DNA-repair rates (significant differences were noted for sex and
21 polymorphisms in *GSTM1*, *XRCC1*, and *XPC* genotypes), differences in the levels and
22 duration of exposure, and the small sample size.

23 The results of 13 studies evaluating DNA damage in workers with high levels of styrene
24 exposure from fiberglass-reinforced-plastics production, boat building, or hand
25 lamination are summarized in Table 5-11. Twelve studies used peripheral blood
26 lymphocytes, and one study (Migliore *et al.* 2002) used sperm cells. All studies included
27 exposure measures — either styrene in air, mandelic acid (or mandelic acid plus
28 phenylglyoxylic acid) in urine, or both. In three studies (Godderis *et al.* 2004, Laffon *et*
29 *al.* 2002a, Maki-Paakkanen *et al.* 1991), the authors estimated styrene concentrations in
30 air from urinary mandelic acid levels.

1 Higher levels of DNA damage were found in styrene-exposed individuals than in controls
2 in all of the studies using the DNA unwinding assay (Brenner *et al.* 1991, Maki-
3 Paakkanen *et al.* 1991, Shamy *et al.* 2002, Walles *et al.* 1993) and in 6 of the 8 studies
4 using the comet assay (Buschini *et al.* 2003, Laffon *et al.* 2002a, Migliore *et al.* 2002,
5 Somorovská *et al.* 1999, Vodicka *et al.* 1995, Vodicka *et al.* 1999). No increase in single-
6 strand breaks was reported in a study that used nick translation (Holz *et al.* 1995).

7 Some studies found a significant correlation between DNA damage and markers of
8 styrene exposure ($r = 0.753$, $P < 0.01$ for urinary mandelic acid; $r = 0.601$, $P < 0.01$ for
9 urinary phenylglyoxylic acid (Shamy *et al.* 2002); $r = 0.470$, $P = 0.031$ for styrene
10 concentration at the workplace, and $r = 0.545$, $P = 0.036$ for styrene concentration in the
11 blood (Vodicka *et al.* 1999); see Section 5.5.3.1 for a description of the studies by
12 Vodicka *et al.*). Significant correlations also were found between DNA damage and *N*-
13 terminal hemoglobin adducts (partial $r = 0.23$, $P = 0.010$) (Godderis *et al.* 2004) or O⁶-
14 guanine DNA adducts ($r = 0.719$, $P = 0.001$) (Vodicka *et al.* 1999). Walles *et al.* (1993)
15 reported that single-strand breaks correlated significantly with increasing exposure when
16 measured at the end of a shift but not at the beginning of a shift. [This study did not use
17 controls.] Single-strand breaks in sperm cells did not correlate with urinary markers in a
18 study of hand laminators in Italy, but the urinary samples were taken on a different day
19 than the semen samples. In contrast to these findings, (Vodicka *et al.* 2004a) found that
20 single-strand breaks correlated negatively with most markers of styrene exposure
21 ($r = -0.350$, $P = 0.007$ for styrene in blood; $r = -0.402$, $P = 0.01$ for urinary mandelic
22 acid; $r = -0.403$, $P = 0.001$ for urinary phenylglyoxylic acid; and $r = -0.375$, $P = 0.003$
23 for urinary 4-vinylphenol conjugates). As discussed above, Vodicka *et al.* suggested that
24 styrene exposure might induce more efficient repair of single-strand breaks because of a
25 positive correlation between DNA repair capacities and markers of styrene exposure.

26 All of the studies of DNA damage obtained information on the smoking history of the
27 subjects, and two studies (Shamy *et al.* 2002, Vodicka *et al.* 1995) noted that no smokers
28 were included in their exposed or control groups. Of the remaining 11 studies, 3 found
29 that smoking had potentially confounding effects on levels of DNA damage. Brenner *et*
30 *al.* (1991) observed that the number of cigarettes smoked per day significantly increased

1 the number of single-strand breaks in the exposed group [no smokers were included in
2 the control group]. Walles *et al.* (1993) reported that smoking increased single-strand
3 breaks in samples taken at the end of a shift, and Laffon *et al.* (2002a) found an increase
4 in DNA tail length in the comet assay for smokers in the exposed group. No other
5 potential confounders were reported to have a significant effect; however, not all studies
6 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 <i>et al.</i> 1991 U.S.	air: 0.6–44 urine: 244	fiberglass-reinforced boat building 2.7 yr 14 exposed 9 controls	<i>DNA unwinding, hydroxylapatite sep.</i> 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 <i>et al.</i> 1991 NR	air: [70] ^b urine: 9.4 mmol/L	reinforced-plastics production 6.7 yr 9 exposed 8 controls	<i>DNA unwinding, hydroxylapatite sep.</i> 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 <i>et al.</i> 1993 Sweden	air: 0.4–20 urine: ND–261	plastics factory 0–25 yr 17 exposed 0 controls	<i>Alkaline elution</i> normalized area above elution curve <i>Time relative to shift</i> before 33 x 10 ⁻³ /h* end 41 x 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 <i>et al.</i> 1995 Former German Democratic Republic	air: [0.017–0.82] urine: 43.9 ± 31.5	styrene production plant 18 yr 25 exposed 25 controls	<i>Nick translation</i> 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 <i>et al.</i> 1995 Bohemia	air: [21–28] urine: 146 ± 77	hand laminators 9 yr 9 exposed 15 controls	<i>Comet</i> : tail moment <i>Abnormal cells</i> exposed 5.50 ± 3.04* control 1.00 ± 3.41 tail length and % DNA in tail also significant <i>Total cells</i> : NS	All subjects were nonsmokers
Vodicka <i>et al.</i> 1999 Bohemia	air: [16–38] urine: 161–351	hand laminators 7.2 yr 13 exposed 13 controls	<i>Comet</i> : 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 <i>et al.</i> 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	<i>Comet</i> : % 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 <i>et al.</i> 2002a Spain	air: 17–19 ^b urine: 313–353	fiberglass-reinforced-plastics factory 17 yr 14 exposed 30 controls	<i>Comet</i> : 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 <i>GSTM1</i> and <i>GSTT1</i> 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 <i>et al.</i> 2002 Egypt	air: NR urine: 90–170	reinforced plastic plant 20 yr 26 exposed 26 controls	<i>DNA unwinding assay</i> <i>hydroxyapatite separation</i> % DNA with SSBs, median (range) exposed 40 (22–65)** control 10 (6.5–13) <i>Exposure-response with urinary markers (r)</i> 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 <i>et al.</i> 2003 Italy	air: 36.8 ± 0.7 urine: 206 ± 2.4^c	polyester resin production; glass-fiber-reinforced plastic manufacture 8.5 yr 48 exposed 14 controls	<i>Comet</i> : tail moment 99th percentile exposed $34.1 \pm 14.0^{***}$ control 12.4 ± 4.9 median: NS	Controls were of comparable age and sex
Vodicka <i>et al.</i> 2004a Bohemia	air: $[19 \pm 13.1]$ urine: 206 ± 2.4^c	fiberglass-reinforced plastic manufacture 4 yr 86 exposed 16 plant controls 26 external controls	<i>Comet</i> : SSBs per 10^9 Da exposed 0.29 ± 0.21 plant control 0.57 ± 0.26 external control 0.53 ± 0.26 correlation (<i>r</i>), <i>P</i> -value <i>Blood</i> : styrene: $-0.350, 0.007$ <i>Urinary</i> : 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 <i>et al.</i> 2004 Belgium	air: 9.5 ± 9.6^b urine: 202 ± 148	laminators 14.2 yr 44 exposed 44 controls	<i>Comet</i> : % 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 <i>et al.</i> 2002 Italy	air: NR urine: 202 ± 148	hand laminators 9.2 years 46 exposed 27 controls	<i>Comet</i> : tail moment exposed 1.5 ± 0.6*** control 0.8 ± 0.4 % DNA in tail: significant*** <i>Exposure-response with urinary markers</i> : 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/m³ to ppm (1 mg/m³ ≈ 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.

1 5.4.4.3 Mutations

2 Studies evaluating mutation frequency (for *HPRT* or glycophorin A [*GPA*] genes) in
3 styrene-exposed workers are summarized in Table 5-12. Mutations at the *HPRT* locus
4 may be associated with a number of other factors (e.g., different types of T cells with
5 different lifespans, host polymorphisms affecting metabolism and DNA repair, and
6 background exposures, such as food intake or smoking) (Vodicka *et al.* 1995).

7 Tates *et al.* (1994) reported that *HPRT* mutation frequency was higher among 46 workers
8 from the former German Democratic Republic who had been exposed to styrene and
9 dichloromethane than in 5 controls [mutation frequency could be measured for only 5 of
10 23 controls]. Controls were matched by age, sex, and smoking status [No other
11 information was provided on the control subjects.].

12 A series of studies measuring *HPRT* mutation frequency in a relatively small number of
13 lamination workers from Bohemian hand-lamination factories (with up to six samplings
14 for each individual) was conducted during a three-year period by Vodicka and co-
15 workers and reported in two publications (Vodicka *et al.* 1995, 1999) (see also the
16 description of this population in Section 5.4.4.1), and another study of workers with
17 differing levels of exposure to styrene in these factories was published by Somorovská *et*
18 *al.* (1999) and Vodicka *et al.* (2001a). Results for *HPRT* mutation frequency from all of
19 these studies were also summarized and reanalyzed in two reviews of styrene
20 genotoxicity by Vodicka *et al.* (2002a, 2003). The 2003 analysis of all of the samplings
21 from this population (Vodicka *et al.* 1995, 2001a, 1999) found a higher *HPRT* mutation
22 frequency in styrene-exposed workers (19.8 ± 20.1 per 10^6 cells) than in controls ($14.9 \pm$
23 7.7), but the difference was not significant ($P = 0.656$). Some of the individual samplings
24 did result in significant differences between exposed workers and controls; *HPRT*
25 mutation frequency was significantly higher in workers than external controls but not
26 factory controls in one of four samplings in the 1995 study and in the sixth sampling in
27 the 1999 study ($P = 0.039$) (Vodicka *et al.* 1999). In the 2001 study of these workers,
28 *HPRT* mutations were higher in styrene-exposed workers than controls, though not
29 significantly so. Although Vodicka *et al.* (2003) reported that their analysis of all data
30 from the hand-lamination workers did not show a significant difference between exposed

1 workers and controls, they did find a significant correlation ($r = 0.588$, $P = 0.001$)
2 between *HPRT* mutation frequency and cumulative exposure [the product of an arbitrary
3 exposure level and years of employment]. Individual studies had also shown significant
4 correlations between *HPRT* mutation frequency and styrene concentration in air, styrene
5 concentration in blood, urinary mandelic acid, hemoglobin adducts, years of employment,
6 age of employees, or heterozygosity in the CYP2E1 and GSTP1 genes (Vodicka *et al.*
7 2001a, Vodicka *et al.* 1999). None of the studies reviewed reported a significant
8 correlation between DNA adducts and *HPRT* mutation frequency.

9 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
11 unexposed individuals matched for age, gender, and smoking status. All exposed and
12 control subjects had the *GPA* M/N heterozygous genotype. *GPA* variant frequencies
13 reflecting allele loss (ϕ/N) or allele loss and duplication of the remaining allele (N/N)
14 were examined. Styrene exposure did not affect ϕ/N frequency, but N/N frequency was
15 higher among workers than controls ($P = 0.058$). When workers were classified into low-
16 and high-exposure groups, the N/N frequency was significantly higher ($P = 0.036$) in the
17 high-exposure group, based on a multivariate analysis of covariance model. However, the
18 significant difference was seen only when one individual in the high-exposure group with
19 an exceptionally low value was excluded; when that individual was included in the
20 analysis, no difference was found. Significant increases in both ϕ/N and N/N frequencies
21 also were seen among active smokers, but the analysis for styrene exposure was adjusted
22 for smoking. The authors concluded that occupational exposure to styrene in the
23 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 <i>et al.</i> 1994 Production of containers & boards with polyester resin (19%–33% styrene) <i>HPRT</i>	5 control (NR)	0	0	8.6 ± 1.2	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
	24 group I (16/8)	19 (4–31)	[20 (0–140)]	15.9 ± 21.1	
	22 group II (9/13)	20 (8–31)	[12 (0–34)]	12.7 ± 6.8	
	46 total (25/21)	20 (4–31)	[16 (0–140)]	14.3 ± 15.7	
Bohemia (some overlap in subjects among studies; hand laminators from same plants)					
Vodicka <i>et al.</i> 1995 Hand-lamination plant <i>HPRT</i>	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 <i>et al.</i> 1999 Hand-lamination plant <i>HPRT</i>	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 <i>et al.</i> 1999, Vodicka <i>et al.</i> 2001a Plastics lamination plant (hand-lamination workers) <i>HPRT</i>	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 (<i>P</i> = 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 <i>et al.</i> 1996 Reinforced-plastics workers GPA ϕ /N	47 control (23/24)	0	0	8.1 ^c	Multivariate analysis of data adjusted for age, gender, smoking status, and styrene exposure for both ϕ /N and N/N
	47 all exposed (23/24)	8.5 ± 6.6	[36 ± 27]	7.2 ^c	
	28 highest exp. (NR)	NR	[≥ 20]	7.6 ^c	
	19 lowest exp. (NR)	NR	[0.2–19]	7.5 ^c	
GPA N/N	47 control (23/24)	0	0	5.0 ^c	Difference nonsignificant for high-exposure group when 1 subject with an exceptionally low value was included
	47 all exposed (23/24)	8.5 ± 6.6	[36 ± 27]	6.3 ^c	
	28 highest exp. (NR)	NR	[≥ 20]	7.2 ^{c*}	
	19 lowest exp. (NR)	NR	[0.2–19]	6.0 ^c	

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/m³ to ppm (1 mg/m³ ≈ 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.

1 *5.4.4.4 Cytogenetic markers*

2 Cytogenetic markers include chromosomal aberrations, micronuclei, and SCE. The
3 cytogenetic effects of occupational exposure to styrene have been reviewed (Bonassi *et*
4 *al.* 1996, Cohen *et al.* 2002, Henderson and Speit 2005, Vodicka *et al.* 2006b).
5 Guidelines for monitoring of genotoxic effects in humans are available in Albertini *et al.*
6 (2000). Many of the studies reviewed in this section evaluated more than one cytogenetic
7 marker; however, results are discussed separately for each marker.

8 Most of the reviewed studies used questionnaires to gather information on exposed and
9 referent population characteristics such as age, sex, socio-economic status, disease status,
10 smoking habits, vaccinations, and past or current exposures to other clastogenic agents,
11 including X-rays.

12 *Chromosomal aberrations*

13 Structural chromosomal aberrations were measured in lymphocytes from styrene-exposed
14 workers in 31 studies. Details on the study population, exposure levels, study design, and
15 results for structural chromosomal aberrations are summarized in Table 5-13 and the
16 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.)	Gaps ^c	Breaks ^c	
Meretoja <i>et al.</i> 1977 (Finland)	Polyester plastic manufacturing workers — 3 plants (laminators) (0.6–8.5 yr)	Exposed 10 Controls 5	NR	[721 (23–3,257)]	Gaps ^c [0.3] [0.2]	Breaks ^c [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 <i>t</i> -test
Meretoja <i>et al.</i> 1978a (Finland)	Polyester plastic manufacturing workers — 2 plants (laminators) (1–15 yr)	<i>Exposed</i> 1976 16 1977 10 <i>Controls</i> 6	NR (≤ 300) NR (≤ 300)	[570 (23–3,257)] [329 (52–1,646)]	<u>Total</u> 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 <i>t</i> -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)	<i>Exposed</i> group A 5 group B 12 group C 14 <i>Controls</i> 20	NR NR (0.01–0.53) ^f NR (50–300) ^g	(mg/L) NR (19–40) NR (< 5–100) NR (102→1,500)	<u>Total</u> 3.8 5.1 9.2* 5.5	<u>w/o Gaps</u> 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 <i>P</i> values not identified
Hogstedt <i>et al.</i> 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		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 <i>et al.</i> 1980 (Sweden)	Reinforced plastic boat factory workers (0.3–12 yr)	<i>Exposed</i> total 36 high 14 low 22 <i>Controls</i> 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		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: <i>t</i> -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
					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: (<i>r</i> = 0.58): significant High-dose group: not significant		

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 <i>et al.</i> 1980 (Germany)	Polyester resin processing workers (4–27 yr)	<i>Exposed</i> 24 laboratory pilot plant <i>Controls</i> 24	6 (1–11.5) 58 (0.7–178)	(mg/L) 0–320	<u>Gaps</u> 5.1 NR NR 3.8	<u>w/o Gaps</u> 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 <i>et al.</i> 1981 (Japan)	Group 1: Reinforced-plastics boat workers Group 2: Polyester resin board workers (0.6–9.3 yr)	<i>Group 1</i> exposed 9 controls 5 <i>Group 2</i> 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)	<u>Total</u> 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: <i>t</i> -test or Chi-square
(Watanabe <i>et al.</i> 1983) (Japan)	Male fiber-reinforced-plastics boat factory workers in 2 workshops	<i>Exposed</i> total 18 group A 10 group B 8 <i>Controls</i> 6	40–50 (NR)	(mg/L) 332 (0–1,041) 399 (0–1,041) 249 (8–999)	<u>Total</u> 6.5 ⁱ 6.6 6.4 4.7	<u>w/o Gaps</u> 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)				<u>Exposure response</u> <i>Styrene air levels, urinary levels and exposure duration: no relationship</i>		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 <i>t</i> -test (2-tailed)
Dolmierski <i>et al.</i> 1983 (NR)	Laminators (1–30 yr)	Exposed 30 Controls 2	NR [< 23]	NR	<u>Gaps</u> 26.9* 14.4	<u>Breaks</u> 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
					<u>Exposure response</u> <i>Length of employment: no correlation</i>		

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 <i>et al.</i> 1983, Camurri <i>et al.</i> 1984 (Italy)	Reinforced unsaturated polyester resin manufacturing workers in 9 plants (1–22 yr)	Plant 1	3	NR [7–9]	(mg/L) NR (45–75)	<u>Total</u>	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 <i>t</i> -test, linear regression
		Controls	3			30**	
		Plant 2	4	NR [16–23]	NR (65–133)	9	
		Controls	4			23***	
		Plant 3	4	NR [23–34.5]	NR (170–694)	8	
		Controls	6			24***	
		Plant 4	5	NR [34.5–46]	NR (151–786)	8.4	
		Controls	6			26***	
		Plant 5	6	NR [46–57.5]	NR (340–671)	8.4	
		Controls	6			32***	
		Plant 6	2	NR [57.5–69]	NR (615–777)	7	
		Controls	2			39**	
		Plant 7	2	NR [69–80.5]	NR (489–828)	6.5	
		Controls	2			37**	
Plant 8	4	NR [80.5–92]	NR (504–909)	8			
Controls	2			25***			
Plant 9	7	NR (> 92)	NR (389–1,108)	4			
Controls	4			44***			
						<u>Exposure response (all subjects)</u> <i>Styrene air concentrations and urinary metabolites:</i> Linear increase ($P < 0.01$) in 1983 study but not in 1984 study	
Hansteen <i>et al.</i>	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.) ^j	Gaps	w/o Gaps	
1984 (Norway)	reinforced polyester plant workers (NR)	<i>Exposed</i> Total 18 group 1 11 group 2 7 <i>Controls</i> 9	13.2 (2–44) 7.5 (2–13) 22.3 (14–44)	NR (200–1,200) ^j	19.5*** 17.6 22.4*** 13.4	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; <i>P</i> values reported for total group for gaps only
Nordenson and Beckman 1984 (Sweden)	Glass-fiber reinforced polyester plant workers (1–26 yr)	<i>Total</i> exposed 15 controls 13 <i>Smokers</i> exposed 4 controls 3 <i>Nonsmokers</i> exposed 11 controls 10	24 (NR)	(mM) NR (< 2)	(CA/100 cells)		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
					<u>Total</u> 2.8 2.7 4.6 2.7 2.1 2.7	<u>Breaks^k</u> [0.4] [0.4] [0.6] [0.3] [0.3] [0.4]	
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.)	Gaps	Breaks	
de Jong 1985 (Netherlands)	oxide and styrene manufacturing workers				1.33 4.36* 0.84 1.32 0.78	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
Pohlova and Sram 1985 (Czech Republic)	Plant A: polystyrene vessels Plant B: sports boats, plastics (1–11 yr)	<i>Plant A</i> June 36 November 34 Controls 19 <i>Plant B</i> June 22 Controls 22 January 19 Controls 17	[16–35 (1.4–226)] [NR (9–126)]	($\mu\text{g/L}$) 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 1 st sampling to 2 nd 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 2 nd sampling in exposed workers No significant differences found for drug intake, gender, and smoking Statistics: Student's <i>t</i> -test
Maki- Paakkanen 1987 (Finland)	Reinforced- plastics workers (mainly laminators) (1–25 yr)	Exposed	21	[23 (8–60)]	(mM) 2.0 (0–7.3)	<u>Total</u> 4.5	<u>w/o Gaps</u> 3.0	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 <i>t</i> -test
Forni <i>et al.</i> 1988 (Italy)	(A) Reinforced- plastics workers (18.8 yr) (B) Plastic boat manufacturing workers (4.5 yr)	<i>Factory A</i> exposed	32	NR [0.4–57]	NR	<u>Total</u> 3.2	<u>w/o Gaps</u> 2.3*	All factory workers were male Controls lived in the same industrial area (gender not identified) matched for age and smoking
		controls	32			2.9	1.6	
		<i>Factory B</i> exposed	8	NR [9.4–45.5]		4.3	2.5	Subjects not exposed to other known genotoxic agents (radiation, chemicals, drug intake, and
		controls	8			2.9	1.5	

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 <i>et al.</i> 1988 (Czech Republic)	Laminated plastic shop workers (11 yr)	Exposed 11 Controls 11	[58 (27–134)]	($\mu\text{L}/\text{mM}$) ^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 <i>t</i> -test
(Hagmar <i>et al.</i> 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)				<u>Exposure response</u> <i>No association with years of employment</i>		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 <i>et al.</i> 1991 (Finland)	Reinforced plastic workers (controls from research institute) (smokers – 6.4 yr, non-smokers – 7.2 yr)	<i>Total</i>	[~ 70] (NR) ^o	(mM) 9.4 (< 1–21.5)	<u>Total</u>	<u>w/o Gaps</u>	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 <i>t</i> -test (one-tailed)
		exposed 17 controls 17 <i>Smokers</i> exposed 11 controls 11 <i>Nonsmokers</i> exposed 6 controls 6			5.3 5.7 4.9 6.8 6.0* 3.7	3.0 3.1 3.0 3.4 3.0 2.7	
					<u>Exposure response</u> <i>Exposure duration: r = 0.59, P < 0.02</i>		

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 <i>et al.</i> 1991 (Finland)	Reinforced plastic industry workers from 32 enterprises (NR)	<i>Past exp. (index pt)</i>	43 (5–182)	(mM) 2.2 (NR) ^{i,p}	<u>w/o Gaps</u> 1.8 1.9 2.4 1.4 1.8 1.6 NR NR	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
		<i>Laminators</i> low 22 high 28				
Tomanin <i>et al.</i> 1992 (Italy)	Polyester resin workers at 2 factories producing fiberglass tanks (1–18 yr) or fiberglass boats (1.5–15 yr)	<i>Factory 1 (low)</i> exposed 7 controls 7	NR [4.8–23]	186 (46–345)	<u>w/o Gaps</u> 1.4 1.4	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
		<i>Factory 2 (high)</i> exposed 11 controls 11	NR [26–100]	725 (423–1,325)	3.0* 0.8 <u>Exposure response</u> <i>Exposure duration:</i> 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.)	Total	w/o Gaps	
Anwar and Shamy 1995 (Egypt)	Reinforced- plastics plant workers (5–22 yr)	Exposed 18 Controls 18	NR	328 (145–1,204) 50 (22–92)	6.1* 3.4	4.0* 1.4	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 <i>et al.</i> 1999 (Lithuania)	Carpet plant workers (2 mo–21 yr) Plasticware plant workers (2 mo–25 yr)	<i>Exposed</i> carpet 79 plastics 97 <i>Controls</i> 90	NR [0.03–0.32] NR [1–1.4]	NR NR	(CA/100 cells) w/o Gaps 3.8* 4.2* 1.7		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: <i>t</i> -test on average square root-transformed data, ANOVA
Somorovská <i>et al.</i> 1999 (Bohemia)	Plastics hand- lamination plant workers (14 yr)	<i>Exposed</i> total 44 low 15 medium 12 high 17 <i>Controls</i> 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.)		
					<u>Exposure response</u> <i>Styrene in air:</i> $r = 0.43$ ($P = 0.001$) <i>Blood levels:</i> $r = 0.41$ ($P = 0.001$) <i>Exhaled air:</i> $r = 0.5$, ($P < 0.001$) <i>Exposure duration:</i> $r = 0.55$ ($P < 0.001$)	chromosome-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 <i>et al.</i> 2001 (Germany)	Boat building workers (8.7 yr)	<i>Total</i> exposed 14 controls 7 <i>Smokers</i> exposed 4 controls 3 <i>Nonsmokers</i> exposed 10 controls 4	NR [<23]	NR	(CA/100 cells) <u>Exchanges</u> 0.22 ^d 0.14 0.25 0.54 0.23 0.10	Controls were from a wood manufacturing training center, not matched for smoking (higher in controls) 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 <i>et al.</i> 2002 (Hungary)	Oil refinery workers (NR)	Exposed 10 Controls 25	NR	NR	<u>w/o Gaps</u> 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 <i>t</i> -test
Vodicka <i>et al.</i> 2004a (Czech Republic)	3 Reinforced- plastic lamination plants: (A: 3.4 yr) (B: 5.6 yr) (C: 2.5 yr)	<i>Exposed</i> total 86 plant A 35 plant B 31 plant C 20 <i>Controls</i> plant 16 external 26	[19] (NR) [26] (NR) [11] (NR) [19] (NR)	497 (NR) ^j 798 (NR) 270 (NR) 308 (NR) 42	<u>w/o Gaps</u> 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. <u>Exposure response</u> <i>No correlation with any marker of styrene exposure</i>	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 <i>et al.</i> 2004c (Slovak Republic)	3 Groups of tire plant workers (1: 19.4 yrs) (2: 19.1 yrs) (3: 12.1 yrs)	<i>Exposed</i> group 1 53 group 2 41 <i>Controls</i> 16	NR [1.9–3.0] NR (NR)	NR	<u>Total</u> 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.)								
							<p>Confounding factors (such as X-rays, drug use, dietary and life style-factors were recorded in questionnaires</p> <p>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</p> <p>Workers exposed to 1,3-butadiene, PAHs, and sulfur dioxide</p> <p>Statistics: Mann-Whitney U-test</p>					
Migliore <i>et al.</i> 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	<table border="1"> <tr> <td>Total</td> <td>w/o Gaps</td> </tr> <tr> <td>3.3</td> <td>2.4</td> </tr> <tr> <td>3.6</td> <td>2.5</td> </tr> </table> <p><u>Exposure response</u> <i>Chromosomal type CA (without gaps)</i> 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: $r = 0.306$, $P = 0.058$ <i>Chromatid type CA</i> Airborne styrene, MA + PGA, 4-VPT – no correlation PHEMA: $r = 0.342$, $P = 0.033$</p>	Total	w/o Gaps	3.3	2.4	3.6	2.5	<p>Total population included 95 exposed workers and 98 controls. CA analysis not conducted in all subjects.</p> <p>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</p> <p>Subjects interviewed for personal, occupational, and medical history (X-rays, viral infections and inflammatory disease, drug use)</p> <p>CA without gaps were higher in smokers but were not related to gender.</p> <p>CAs defined as chromatid and chromosomal type aberrations.</p> <p>Statistics: Multifactorial ANOVA and first-order regression</p>
Total	w/o Gaps											
3.3	2.4											
3.6	2.5											

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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/m³ to ppm (1 mg/m³ 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/m³ (10 ppm) except on two occasions where concentrations of 91 and 202 mg/m³ (~21 and 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.

1 [In general the studies (N = 31) of chromosomal aberrations in styrene-exposed workers
2 were limited by a small number of subjects and potential confounding from other
3 workplace exposures.] Most studies included 25 or fewer subjects per group, but some
4 studies (Andersson *et al.* 1980, Dolmierski *et al.* 1983, however there were only 2
5 controls) (Artuso *et al.* 1995, Forni *et al.* 1988, Pohlova and Sram 1985, Somorovská *et*
6 *al.* 1999, Tates *et al.* 1994, Vodicka *et al.* 2004c) had somewhat larger populations
7 (between 30 and 50 in the total exposed or controls) and five studies had populations
8 between 75 and 100 (Lazutka *et al.* 1999, Migliore *et al.* 2006b, Sorsa *et al.* 1991, van
9 Sittert and de Jong 1985, Vodicka *et al.* 2004c).

10 [The exposed and referent populations were usually matched for age, gender, and
11 smoking habits.] Some studies that did not use matched subjects controlled for variables
12 (such as age, gender, and smoking habits) in the analysis or reported that age, smoking,
13 and gender distribution were similar between groups. Studies that did not meet these
14 criteria include Thiess *et al.* (1980) (although the authors stated that smoking information
15 was recorded), Sorsa *et al.* (1991) [not clear whether smoking and age were controlled for
16 in the dose-response regression analysis], Biró *et al.* (2002) (smoking and gender differed
17 between exposed and controls but ages were similar), Oberheitmann *et al.* (2001) (similar
18 ages but smoking differed between exposed and controls) and Vodicka *et al.* (2004c).

19 [None of these studies found an association between chromosomal aberrations and
20 styrene exposure, see below.] Several studies evaluated the effects of potential
21 confounders such as smoking, age, and gender on aberration frequency. Of the 13 studies
22 that evaluated smoking, 11 (Andersson *et al.* 1980, Artuso *et al.* 1995, Biró *et al.* 2002,
23 Camurri *et al.* 1983, 1984 [considered as one study], Lazutka *et al.* 1999, Maki-
24 Paakkanen 1987, Meretoja *et al.* 1978a, Pohlova and Sram 1985, Somorovská *et al.* 1999,
25 Tates *et al.* 1994, Tomanin *et al.* 1992) reported that smoking did not affect, or was not
26 correlated with an increase in chromosomal aberrations; one study (Maki-Paakkanen *et*
27 *al.* 1991) found higher chromosomal aberrations in smokers in the control group than
28 non-smoking controls, and another study (Sorsa *et al.* 1991) reported a positive
29 correlation between chromosomal aberration frequency and smoking. Conflicting results
30 were found for the two studies that evaluated age; Sorsa *et al.* reported that age was
31 correlated with chromosomal aberration frequency; however, Lazutka *et al.* did not find

1 an effect. One study (Somorovská *et al.* 1999) reported that chromosomal aberration
2 frequency was higher in females compared with males; however, Pohlová and Srám did
3 not find any differences related to gender.

4 [Common study quality issues, which are related to the measurement of chromosomal
5 aberrations included cells cultured too long for the peak period of chromosomal
6 aberrations, inadequate number of metaphases scored per individual, or incomplete
7 exposure data. These potential quality issues are identified in the “Comments” column in
8 Table 5-13. Most of the studies examined at least 100 metaphases per person; however,
9 current guidelines recommend a minimum of 200 metaphases per subject (Albertini *et al.*
10 2000). Studies that scored fewer than 100 metaphases per person are identified in the
11 “Comments” column. Several studies used cell-culture times that were longer than ideal
12 (i.e., comprising mostly second division cells). In some cases the authors stated that the
13 longer culture times were chosen so that a larger number of mitotic cells would be
14 available for scoring.]

15 [It is difficult to compare results across studies because the studies were not consistent in
16 data reporting]. Some studies reported the percentage of cells per subject with
17 chromosomal aberrations, while others reported the mean number of aberrations per 100
18 cells. Studies also varied in the type and description of the aberrations reported; some
19 only gave total estimates, whereas others reported the frequency of specific types of
20 chromosomal aberrations (e.g., gaps, breaks, exchanges) or general categories of
21 chromosomal aberrations (e.g., with or without gaps). The data in Table 5-13 include the
22 most comprehensive measures of chromosomal aberrations reported by the study authors
23 and are identified in the “Results” column. When available, information is presented for
24 total chromosomal aberrations without gaps.

25 One study (Oberheitmann *et al.* 2001) measured exchange-type chromosomal aberrations
26 and did not find a significant increase in styrene-exposed workers compared with
27 controls. [The study was limited by small numbers of subjects and unmatched controls
28 (wood manufacturing industry) for smoking (more controls than exposed smoked).]
29 However, after X-ray challenge, the rate of exchange type aberration frequency was

1 higher in exposed compared with historical controls (N = 2). The response was correlated
2 with cumulative lifetime exposure to styrene but not current exposure (see Section
3 5.4.4.2).

4 Of the remaining 30 studies, 17 studies — Meretoja *et al.* (1978a, 1977), Fleig and Thiess
5 (1978) (high exposure subgroup), Högstedt *et al.* (1979), Andersson *et al.* (1980),
6 Dolmierski *et al.* (1983), Camurri *et al.* (1983, 1984), Hansteen *et al.* (1984), Pohlová
7 and Srám (1985) (only for one of two plants, and only for one of two samplings), Forni *et*
8 *al.* (1988), Mäki-Paakkanen *et al.* (1991) (nonsmokers only), Tomanin *et al.* (1992) (high
9 exposure factory), Tates *et al.* (1994), Artuso *et al.* (1995), Anwar and Shamy (1995),
10 Lazutka *et al.* (1999), and Somorovska *et al.* (1999) — reported a significant increase in
11 the frequency of chromosomal aberrations in the exposed population (or subgroup of
12 exposed workers) compared with the controls. [The studies were not consistent in the
13 types of aberrations found to be elevated (see Table 5-13).] Three of these studies found
14 significant increases in gaps only (Dolmierski *et al.* 1983, Hansteen *et al.* 1984, Pohlova
15 and Sram 1985). [Findings reported by Dolmierski *et al.* were limited by the small
16 numbers of controls (N = 2), and findings in some other studies were limited by potential
17 confounding from other occupational exposures.] Workers in the study reported by Tates
18 *et al.* were also exposed to dichloromethane. A positive correlation between styrene
19 exposure (TWA) and chromosomal aberrations was found in one of two exposed
20 subgroups but not the pooled population; however, positive correlations were also found
21 between dichloromethane exposure (TWA) and chromosomal aberrations in that
22 subgroup as well as the total exposed subjects. Workers in the study reported by Lazutka
23 *et al.* were exposed to higher levels of phenol and formaldehyde than styrene, [but no
24 dose-response analysis was performed]. The authors stated that the literature on
25 chromosome damage by occupational exposure to formaldehyde is not consistent and no
26 literature was available on the genotoxic effects of environmental exposure to phenol.
27 Workers in other studies were often exposed to other agents such as peroxides, methylene
28 chloride, and acetone.

29 [In general, “positive” results were observed in studies with higher levels of exposure or
30 in the high-exposure subgroup. An exception is the Lazutka *et al.* study (exposure

1 between 0.03 and 1.4 ppm). This study was also one of the larger studies, and as
2 mentioned above the workers were also exposed to formaldehyde and phenol.] Migliore
3 *et al.* did not report an increase in chromosomal aberration frequency in the exposed
4 workers versus the controls, but did find positive correlations with chromosomal type
5 aberrations (without gaps) with various measures of styrene exposure (styrene air levels
6 and urinary metabolites, and chromatid type aberrations with urinary
7 phenylhydroxyethylmercapturic acids. The average exposure levels were low in this
8 study. In addition to Migliore *et al.*, positive correlations with measures of styrene
9 exposure were also reported by Camurri *et al.* (1983, but not 1984 analysis), Andersson
10 *et al.* (cumulative styrene exposure, increase observed in both low and high subgroup but
11 only significant in the low group), Tates *et al.* (in one subgroup but not in the total
12 population), and Somorovska (air, exhaled air, urinary metabolites, and blood levels).
13 Artuso *et al.* used a multivariate regression model and found a higher RR for
14 chromosomal aberrations for high styrene exposure (RR = 1.71, 95% CI = 1.25 to 2.33)
15 than low styrene exposure (RR = 1.38, 95% CI = 0.98 to 1.94). (Smoking, alcohol
16 drinking, and diagnostic X-rays were not risk factors for chromosomal aberrations in this
17 model). Fleig and Thiess, and Tomanin *et al.* reported higher chromosomal aberrations in
18 the high-exposure subgroup (as assessed by air and urinary metabolite levels) compared
19 with the low-exposure subgroup. Pohlová and Srám measured urinary metabolites and
20 chromosomal aberrations in the same workers (at two different plants) at two different
21 sampling times. Urinary metabolites (see Table 5-13) and chromosomal aberrations
22 increased at the second sampling. At the second sampling, styrene-exposed workers in
23 Plant A had a significant increase ($P < 0.01$) in rates of gaps (from 1st sampling to 2nd
24 sampling), and styrene-exposed workers in Plant B had significantly higher rate of
25 percent aberrant cells (cells with breaks or exchanges).

26 In contrast, the following studies did not find a positive correlation between styrene
27 exposure (either air levels or urinary metabolite level) and chromosomal aberrations:
28 Watanabe *et al.* (1983), Sorsa *et al.* (1991), Anwar and Shamy (1995), and Vodicka *et al.*
29 (2004a). Several studies did not report a significant correlation with exposure duration
30 (Anwar and Shamy 1995, Hagmar *et al.* 1989, Lazutka *et al.* 1999, Tomanin *et al.* 1992,
31 Watanabe *et al.* 1981), while Somorovska *et al.* (1999) and Mäki-Paakkanen *et al.* (1991)

1 did report a significant correlation with exposure duration. [The ability to detect dose-
2 response relationships is limited by small numbers in most studies; studies with the larger
3 numbers of exposed subjects include Migliore *et al.* (2006b), Somorovska *et al.* (1999),
4 Sorsa *et al.* (1991), and Vodicka *et al.* (2004c).]

5 Studies that did not find any significant increases in chromosomal aberrations in workers
6 exposed to styrene include Thiess *et al.* (1980), Watanabe *et al.* (1983, 1981), Nordenson
7 and Beckman (1984), Mäki-Paakkanen (1987), Jablonicka *et al.* (1988), Sorsa *et al.*
8 (1991), Biró *et al.* (2002), and Vodicka *et al.* (2004c, 2004a). van Sittert and de Jong
9 (1985) used pre-exposure measures for the reference group and followed the study
10 population for a couple of years. An increase in chromosomal aberrations was observed
11 for only 1 of the 3 follow-up years. The authors reported that there were no changes in
12 styrene, propylene oxide, and benzene air levels from 1978 to 1981, thus, they did not
13 think the increase in chromosomal aberrations in 1980 was due to occupational exposure,
14 and Migliore *et al.* (2006b) reported a positive dose-response relationship but no
15 significant pair-wise comparison, [which complicates the classification (in terms of
16 positive or negative) of these studies. The study by Sorsa *et al.* had limited
17 documentation on its study population.] Mäki-Paakkanen *et al.* (1991) reported an
18 increase in chromosomal aberration frequency in non-smokers but not in smokers or the
19 total population. Watanabe *et al.* (1983) reported that there was a marginal ($0.05 < P <$
20 0.06) increase in chromosomal aberration frequency in the exposed group compared with
21 the controls. [Most of the “negative” studies had somewhat lower levels of exposures
22 except for Watanabe *et al.* (1983, 1981) and Jablonicka *et al.* (1988).]

23 As mentioned above, there have been several reviews or evaluations of the cytogenetic
24 effects of styrene. Early reviews such as IARC (1994a,b) and Scott and Preston (1994a)
25 are not discussed here since they only include a subset of the available literature to date.
26 Bonassi *et al.* (1996) performed a meta-analysis of 25 (22 with results for chromosomal
27 aberrations) biomonitoring studies of occupational exposure to styrene. The review
28 included all studies up to Artuso *et al.* (in Table 5-13), but did not include two earlier
29 studies, Dolmierski *et al.* (1983) and van Sittert and de Jong (1985). The authors found a
30 positive association (weighted frequency ratio = 2.18, 95% CI = 1.52 to 3.13, weight was

1 assigned to each study depending on its sample variance) between styrene exposure level
2 and chromosomal aberration frequency when exposure levels were dichotomized as
3 greater or less than a threshold value of 30 ppm for an 8-hour time-weighted average
4 (which was the median exposed-group exposure level in the identified studies).

5 Cohen *et al.* (2002) concurred with the Bonassi *et al.* review and also noted that the
6 finding of dose-response relationships makes confounding unlikely. They concluded that
7 there was “compelling evidence” of a positive association between styrene exposure at
8 occupational levels and the frequency of chromosomal abnormalities. However, a review
9 by Henderson and Speit (2005) concluded that the evidence for chromosomal aberrations
10 was conflicting. This review included 27 studies [it did not include Dolmierski *et al.*
11 (1983), van Sittert and de Jong (1985), Vodicka *et al.* (2004c), and Migiliore *et*
12 *al.*(2006a) The authors stated that the Bonassi review did not account for study quality or
13 the type of chromosomal aberrations (such as including gaps).

14 *Micronucleus formation*

15 Details on the study population, exposure levels, study design, and results for structural
16 micronucleus formation are summarized in Table 5-14, and the findings are discussed
17 after the tables.

18 The current guidelines for investigating the frequency of micronucleated blood
19 lymphocytes or epithelial cells in humans are presented by Albertini *et al.* (2000). The
20 cytokinesis-block micronucleus technique is the method of choice [first used in studies on
21 styrene exposure by Mäki-Paakkanen *et al.* 1991]. Cytochalasin B (Cyt-B) is added to the
22 cell culture to block cells from dividing after they have undergone one round of
23 replicative synthesis since mitogen stimulation; such cells are binucleate. Most studies
24 score 1,000 to 2,000 binucleated cells per subject. The results are expressed as the
25 number of micronucleated cells per 1,000 binucleate cells or the percentage of cells with
26 micronuclei (Table 5-14). Some studies also indicated the number of kinetochore-
27 positive/centromere-positive micronuclei and the number of kinetochore-
28 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 <i>et al.</i> 1977 (Finland)	Polyester plastic manufacturing workers from 3 plants (0.6–8.5 yr)	Exposed	10	NR	[721 (23–3,257)]	<u>MN/1,000 cells</u> [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 2 nd division cells scored Statistics: not performed
		Controls	5				
Hogstedt <i>et al.</i> 1983 (Sweden)	Fiberglass- reinforced polyester resin manufacturing workers (1–23 yr)	<i>Preserved cytoplasm</i> exposed	38	13 (1–36)	65 (9–316)	<u>MN/1,000 cells</u> 5.9** 3.6 4.3 3.7 <u>Exposure response</u> <i>Styrene air concentrations, exposure duration, cumulative dose, and urinary mandelic acid: no correlation</i>	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
		<i>Hypotonic treatment</i> exposed	38				
		controls	20				
		controls	20				

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)	<i>Total</i> exposed 12 controls 12 <i>Smokers</i> exposed 4 controls 3 <i>Nonsmokers</i> exposed 8 controls 9	24 (NR)	(mM/L) NR (< 2)	<u>MN/1,000 cells</u> 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)	<i>Total</i> exposed 21 controls 21 <i>Smokers</i> exposed 15 controls 15 <i>Nonsmokers</i> exposed 6 controls 6	[23 (8–60)]	(mM/L) 2.0 (0–7.3)	<u>% cells with MN</u> 1.5 ± 0.1 ^c 1.6 ± 0.1 1.4 ± 0.2 1.6 ± 0.1 1.6 ± 0.2 1.6 ± 0.3 <u>Exposure response</u> <i>Duration, air, or urinary MGA: no correlation</i>	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: <i>t</i> -test (one sided); analysis for exposure response not reported
Hagmar <i>et al.</i> 1989	Glass reinforced polyester plastic	<i>PHA</i> exposed 20	[13 (0.9–127)]	NR	<u>MN/1,000 cells</u> 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 <i>PWN</i> exposed 20 controls 22			4.4 5.9 7.0 <u>Exposure response</u> <i>Employment length:</i> 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 <i>et al.</i> 1991 United States	Reinforced-fiberglass plastic boat workers (2.7 yr)	<i>Exposed</i> total 10 high 4 low 6 <i>Controls</i> 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)]	<u>MN/1,000 cells</u> 10.3 ± 0.4** 10.8 ± 0.6 10.0 ± 0.5 6.5 ± 0.5 <u>Exposure response</u> <i>Positive association by ANOVA, no response observed with continuous variables (air, urinary markers, and cumulative exposure)</i>	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 <i>et al.</i> 1991 (Finland)	Reinforced plastic workers (smokers – 6.4 yr, non-smokers – 7.2 yr)	<i>Total</i> exposed 17 controls 17 <i>Smokers</i> exposed 11 controls 11 <i>Nonsmokers</i> exposed 6 controls 6	[~ 70] (NR) ^f	(mM) 9.4 (< 1–21.5) 11.0 (< 1–16.6) 6.5 (< 1–21.5)	<u>% cells with MN</u> 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 <i>et al.</i> 1991 (Finland)	Reinforced-plastics industry workers from 32 enterprises (NR)	<i>Past exp. (index pts)</i> <i>Laminators</i> low 15 high 13 <i>Other workers</i> low 5 high 6 <i>Controls</i> other factory 31 plastics factory 6 <i>All subjects</i> exposed 50 controls 37	43 (5–182) 11 (1–133)	(mM) 2.2 (NR) ^{g,h}	<u>% cells with MN</u> 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 <u>Exposure response</u>	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)		
						<i>No association with styrene exposure</i>	
Tomanin <i>et al.</i> 1992 (Italy)	Polyester resin workers at 2 factories: fiberglass tanks (1–18 yr) or fiberglass boats (1.5–15 yr)	<i>Factory 1</i> exposed 7 controls 7		NR [4.8–23]	186 (46–345)	<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> <i>Exposure duration:</i> no correlation <i>Urinary MA:</i> weak correlation <i>R</i> = 0.61 (<i>P</i> 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 <i>et al.</i> 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> <i>No association with exposure to styrene after adjusting for gender</i>	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 <i>et al.</i> 1994 (Germany)	Polyester resin/fiberglass plastic products production workers (4–31 yr)	<i>Exposed</i> total group 1 group 2 <i>Controls</i>	46 24 22 23	[16 (0–138)] [20 (0–138)] [12 (0–34)]	NR	<u>MN/1,000 cells</u> 35.1 ± 20*** 32.3 ± 24*** 38.2 ± 13*** 14.3 ± 7.3 <u>Exposure response</u> 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 <i>et al.</i> 1994 (Belgium)	Fiberglass-reinforced plastic pipes and cisterns workers (2.9 yr)	<i>Smokers</i> exposed controls <i>Nonsmokers</i> exposed controls	32 13 17 10	[7 (0.5–25)]	102 (11–649)	<u>MN/1,000 cells</u> 3.28 ± 0.28 ^e 4.32 ± 0.55 3.50 ± 0.34 4.75 ± 0.71 <u>Exposure response</u> Air, urinary MA no correlation	Study consisted of 52 exposed and 24 non-exposed 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)	<u>MN/1,000 cells</u> 6.55 ± 3.47 6.00 ± [2.83] <u>Exposure response</u> <i>Duration, urinary MA: no correlation</i>	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 <i>et al.</i>	Styrene	<i>Total</i>			<u>% cells with MN</u>	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	NR [0.02–> 0.9] NR [≤ 0.01]	13.3–43.9 (NR) ⁱ	1.90 ± 0.78	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: <i>t</i> -test
	controls	25	4.3–5.5 (NR)		1.87 ± 0.71		
		<i>Total</i>					
		exposed	25		13.3–43.9 (NR) ⁱ	<u>% K+ MN</u> 39.4 ± 10.2**	
		controls	25		4.3–5.5 (NR)	31.8 ± 8.2	
		<i>Smokers</i>					
		exposed	17		10–49.4 (NR)	38.3 ± 11.3*	
		controls	13		5.6–6.3 (NR)	30.3 ± 7.9	
		<i>Nonsmokers</i>					
		exposed	8		20.3–32.4 (NR)	42.1 ± 7.4*	
		controls	12		2.8–4.6 (NR)	33.3 ± 8.51	
Karakaya <i>et al.</i> 1997 (Turkey)	furniture workers (10 yr)	<i>Total</i>		30 (20–300)	207 (14–1,482) [§] 12 (0–38) [§]	<u>% cells with MN</u>	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
		exposed	50			1.98 ± 0.50	
		controls	41			2.09 ± 0.35	
		<i>Smokers</i>					
		exposed	36			1.91 ± 0.46	
		controls	29			2.20 ± 0.31	
		<i>Nonsmokers</i>					
		exposed	14			2.18 ± 0.57	
		controls	12			1.82 ± 0.30	
						<u>Exposure response</u> <i>Duration:</i> <i>nonsignificant trend</i>	

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: <i>t</i> -test, Spearman rank correlation, average square root transformation
Laffon <i>et al.</i> 2002a Spain	Fiberglass-reinforced-plastics production workers (≥ 7 yr)	Exposed Controls	14 30	< 20 (NR) ^c	313–353 (NR) ^j	<u>MN/1,000 cells</u> 24.6 ± 1.5** ^e 13.9 ± 0.81 <u>Exposure response</u> <i>Duration: positive correlation (P < 0.001)</i>	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 <i>t</i> -test, Pearson correlation
Teixeira <i>et al.</i> 2004 NR	2 small reinforced-plastics plants (12 yr)	<i>Total</i> exposed controls <i>Men</i> exposed controls <i>Women</i> exposed controls	28 28 18 18 10 10	27 (2–91)	401 (47–1,490) ^g	<u>MN/1,000 cells</u> 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 (<i>P</i> < 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 <i>t</i> -test
Godderis <i>et al.</i> 2004 Belgium	Fiberglass-reinforced plastic workers from 2 plants (7 mo–38 yr)	<i>Lymphocytes</i> MNBC 38 controls 41 MNMC 38 controls 41 <i>Nasal cells</i> 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)	<u>MN/1,000 cells</u> 3.93 ± 2.8* 2.65 ± 1.94 0.71 ± 0.9*** 0.11 ± 0.20 0.52 ± 0.49* 0.23 ± 0.31 <u>Exposure response</u> <i>Employment duration: positive association with MNBC and MNMC</i> <i>Average styrene exposure: positive association with nasal cell MN</i>	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 <i>t</i> -test for nasal cells, bivariate correlation analysis multiple regression analysis (backward, stepwise)
Vodicka <i>et al.</i> 2004a (Czech Republic)	3 Reinforced-plastic lamination plants (A: 3.4 yr)	<i>Exposed</i> total 86 plant A 35 plant B 31	[19] (NR) [26] (NR) [11] (NR) [19] (NR)	497 (NR) ^g 798 (NR) 270 (NR)	<u>% cells with MN</u> 15.1 ± 6.7 17.9 ± 8.1*** 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 <i>Controls</i>	20		308 (NR)	12.5 ± 4.7	(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
		plant external	16 26		42	11.6 ± 4.9 15.6 ± 6.9	
						<u>Exposure response</u> (correlations with styrene exposure) <i>Air levels: P < 0.001</i> <i>Blood levels: P < 0.001</i> <i>Cumulative exposure P < 0.05</i>	
Migliore <i>et al.</i> 2006b (Tuscany and Parma, Italy)	Fiberglass reinforced-plastics workers from 13 plants (1–34 yr)	Exposed Controls	92 98	[8.5 (0.5–123)]	300 (10–1856) ^f	<u>% cells with MN</u> 13.8 ± 0.5*** ^c 9.2 ± 0.42 <u>% cells C+MN</u> 7.43 ± 0.34*** 4.75 ± 0.37 <u>% cells C–MN</u> 5.76 ± 0.3*** 3.20 ± 0.3 <u>Exposure response</u> <i>Air levels: No correlation with total</i>	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 <i>et al.</i> 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/m³ to ppm (1 mg/m³ 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.

1 Micronuclei were measured in peripheral blood lymphocytes from workers exposed to
2 styrene in 20 studies and in nasal epithelial cells in one study (Table 5-14). All but three
3 studies (Anwar and Shamy 1995, Maki-Paakkanen *et al.* 1991, Sorsa *et al.* 1991) scored a
4 minimum of 1,000 cells per subject. [As with the chromosomal aberration studies, data
5 quality issues (e.g., small number of subjects, unmatched controls, and exposure to other
6 clastogenic agents) were identified for several of the studies. About half of the studies
7 included fewer than 25 subjects per group. Most of the studies included control groups
8 matched on one or more of the following variables: age, gender, or smoking. Studies that
9 did not report using matched control groups included Meretoja *et al.* (1977), Hagmar *et*
10 *al.* (1989), Brenner *et al.* (1991), Sorsa *et al.* (1991), and Vodicka *et al.* (2004a). Most of
11 the studies that did not use matched subjects controlled for variables (such as age, gender,
12 and smoking habits) in the analysis or reported that age, smoking, and gender distribution
13 were similar between groups. Only one study (Sorsa *et al.* 1991) did not appear to meet
14 that criterion, although it was not clear whether smoking and age were controlled for in
15 the dose-response regression analysis.]

16 [Most studies evaluated the effects of potential confounders such as smoking, age, and
17 gender on micronuclei, but the results were mixed]. Only three studies (Godderis *et al.*
18 2004, Laffon *et al.* 2002a, Migliore *et al.* 2006b) reported that smoking was correlated
19 with an increase in micronuclei, but nine studies (Brenner *et al.* 1991, Hagmar *et al.*
20 1989, Holz *et al.* 1995, Nordenson and Beckman 1984, Sorsa *et al.* 1991, Tates *et al.*
21 1994, Teixeira *et al.* 2004, Tomanin *et al.* 1992, Van Hummelen *et al.* 1994) did not
22 show a correlation with smoking. Migliore *et al.* (2006b) reported that smoking was not
23 correlated with total micronuclei but was correlated with a decrease in centromere-
24 negative micronuclei. Six studies reported a significant correlation with age (Godderis *et*
25 *al.* 2004, Hagmar *et al.* 1989, Migliore *et al.* 2006b, Sorsa *et al.* 1991, Vodicka *et al.*
26 2004a, Yager *et al.* 1993), but four studies reported no correlation with age (Anwar and
27 Shamy 1995, Brenner *et al.* 1991, Laffon *et al.* 2002a, Van Hummelen *et al.* 1994)
28 [although a non-significant increase with age was reported by Laffon *et al.*]. Four studies
29 (Migliore *et al.* 2006b, Teixeira *et al.* 2004, Vodicka *et al.* 2004a, Yager *et al.* 1993)
30 reported that micronuclei were higher in females compared with males. However,
31 Brenner *et al.* (1991) did not find any differences related to gender.

1 Yager *et al.* (1993) conducted a longitudinal study (without controls) that compared
2 styrene exposure (measured at several times during a one year period) with micronucleus
3 frequency. No correlation was found between styrene exposure (air levels and exhaled
4 air) and micronucleus frequency.

5 Micronuclei were significantly increased in styrene-exposed workers in at least one
6 exposed group in 10 (of the remaining 19) studies (Brenner *et al.* 1991, Godderis *et al.*
7 2004, Hogstedt *et al.* 1983, Holz *et al.* 1995, Laffon *et al.* 2002a, Meretoja *et al.* 1977,
8 Migliore *et al.* 2006b, Nordenson and Beckman 1984, Tates *et al.* 1994, Vodicka *et al.*
9 2004a). Only kinetochore-positive micronuclei [an indicator of aneuploidy] were
10 increased in the study by Holz *et al.* (1995), and Vodicka *et al.* (2004a) reported
11 significantly more micronuclei in a single subgroup of workers (one of three plants). Of
12 the 12 studies that evaluated dose-response relationships, five reported a significant
13 correlation with styrene exposure (Brenner *et al.* 1991, Tates *et al.* 1994 (in one of the
14 subgroups but not the pooled population), Laffon *et al.* 2002a, Godderis *et al.* 2004, and
15 Vodicka *et al.* 2004a). Workers in the study reported by Tates *et al.* were also exposed to
16 dichloromethane; however, no correlation was found between dichloromethane [which is
17 a genotoxin] and micronuclei. Holz *et al.* (1995) attributed the increase in kinetochore-
18 positive micronuclei to exposure to benzene. Workers in other studies were also exposed
19 to other chemicals such as peroxides, organic solvents, acetone (Brenner *et al.* 1991,
20 Hogstedt *et al.* 1983, Laffon *et al.* 2002a, Nordenson and Beckman 1984), but it was not
21 reported whether these chemicals can cause micronuclei.

22 Nine studies did not find increased micronuclei frequency in the exposed groups (Anwar
23 and Shamy 1995, Hagmar *et al.* 1989, Karakaya *et al.* 1997, Maki-Paakkanen 1987,
24 Maki-Paakkanen *et al.* 1991, Sorsa *et al.* 1991, Teixeira *et al.* 2004, Tomanin *et al.* 1992,
25 Van Hummelen *et al.* 1994). The exposed populations in these studies ranged from 7 to
26 50 subjects, and the reported mean styrene concentrations ranged from about 7 to 70
27 ppm, which were similar to those reported for the positive studies. Bonassi *et al.* (1996)
28 conducted a meta-analysis of 10 studies of micronucleus frequency in styrene-exposed
29 workers (Brenner *et al.* 1991, Hagmar *et al.* 1989, Hogstedt *et al.* 1983, Maki-Paakkanen
30 1987, Maki-Paakkanen *et al.* 1991, Meretoja *et al.* 1977, Nordenson and Beckman 1984,

1 Sorsa *et al.* 1991, Tates *et al.* 1994, Tomanin *et al.* 1992) and concluded that the data
2 were inconclusive. Of the 10 studies published since that analysis, five reported positive
3 associations. Cohen *et al.* (2002), noting a general lack of evidence of a significant dose
4 response and inadequate control for potential confounders, concluded that there was no
5 compelling evidence in humans that exposure to styrene was associated with
6 micronucleus formation.

7 *Sister chromatid exchange*

8 Details on the study population, exposure levels, study design, and results for structural
9 sister chromatid exchange (SCE) are summarized in Table 5-15, and the findings are
10 discussed after the tables.

11 SCE scoring is conducted in second-division metaphases and requires DNA replication in
12 the presence of bromodeoxyuridine (BrdU) for two consecutive cell cycles, or at least the
13 first of two consecutive cell cycles (Albertini *et al.* 2000). It is necessary to score 30 to 50
14 second-division metaphase cells to obtain a stable estimate of the mean; however, a
15 minimum of 80 cells is recommended to identify a small proportion (~10%) of high-
16 frequency SCE cells (cells with an abnormally high number of SCEs). Seven studies
17 included subjects that had fewer than 30 metaphases scored (Andersson *et al.* 1980,
18 Brenner *et al.* 1991, Holz *et al.* 1995, Meretoja *et al.* 1978a, Teixeira *et al.* 2004,
19 Watanabe *et al.* 1983, Watanabe *et al.* 1981). Data are recorded as the frequency of SCE
20 per cell and also may include the proportion of high-frequency cells (HFCs). Because
21 baseline levels of SCEs show considerable variation among individuals and between
22 studies, it is difficult to classify subjects into high, medium, or low categories (Albertini
23 *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 <i>et al.</i> 1978a (Finland)	Polyester plastic manufacturing workers (laminators) (1–15 yr)	Exposed	11	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 <i>t</i> -test
Andersson <i>et al.</i> 1980 (Sweden)	Reinforced-plastics boat factory workers (0.3–12 yr)	<i>Exposed</i> total high low <i>Controls</i>	20 6 14 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 <i>t</i> -test
Watanabe <i>et al.</i> 1981 (Japan)	Group 1: Reinforced-plastics boat factory (workshop 1) Group 2: Polyester resin	<i>Group 1:</i> exposed controls <i>Group 2:</i> exposed controls	9 5 7 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 <i>t</i> -test or Chi-square
Watanabe <i>et al.</i> 1983 (Japan)	Fiber reinforced-plastics boat factory workers in 2 workshops (groups A & B) (1 mo–30 yr)	<i>Exposed</i> total 18 group A 10 group B 8 <i>Controls</i> 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 <u>Exposure response</u> <i>total urinary metabolites: r = 0.525, P < 0.05</i>	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, <i>t</i> -test (two-tailed)
Camurri <i>et al.</i> 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	NR [57.5–69]	NR (615–777)	19.6 ± 5.2	occurring at about 250 mg/m ³ [57.5 ppm] Statistics: Student's <i>t</i> -test	
		Control	2			10.3 ± 0.1		
		Plant 7	2	NR [69–80.5]	NR (489–828)	16.1 ± 1.6*		
		Control	2			10.4 ± 0.0		
		Plant 8	4	NR [80.5–92]	NR (504–909)	16.1 ± 0.13**		
		Control	2			9.7 ± 1.5		
		Plant 9	7	NR (>92)	NR (389–1,108)	15.1 ± 0.5***		
		Control	4			8.5 ± 1.1		
Hansteen <i>et al.</i> 1984 (Norway)	Glass-fiber reinforced polyester plant workers (2 groups based on exposure levels) (NR)	<i>Exposed</i> total	18	13.2 (2–44) 7.5 (2–13) 22.3 (14–44)	NR (200–1,200) ^c	6.6		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
		group 1	11			6.9		
		group 2	7			6.0		
		<i>Controls</i>	9			6.5		
Maki-Paakkanen 1987 (Finland)	Reinforced-plastics workers (mainly laminators) (1–25 yr)	Exposed	21	[23 (8–60)]	(mM) 2.0 (0–7.3)	7.6 ± 0.2 ^f 7.4 ± 0.2	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 <i>t</i> -test, analysis for exposure response not reported	
		Controls	21			<u>Exposure response</u> <i>No correlation with exposure extent or duration</i>		
Kelsey <i>et al.</i> 1990	Reinforced-fiberglass plastic boat building	<i>Smokers</i> 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 <i>Nonsmokers</i> 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 <u>Exposure response</u> <i>No increase with styrene air levels or urinary metabolites</i>	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 <i>t</i> -test, ANOVA for exposure response analysis
Maki-Paakkanen <i>et al.</i> 1991 (Finland)	Reinforced plastic workers (smokers – 6.4 yr, non-smokers – 7.2 yr)	<i>Total</i> exposed 17 controls 17 <i>Smokers</i> exposed 11 controls 11 <i>Nonsmokers</i> 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 <i>t</i> -test (one-tailed)
Brenner <i>et al.</i> 1991 (United States)	Reinforced fiberglass plastic boat workers (2.7 yr)	<i>Exposed</i> total 10 high 4 low 6 <i>Controls</i> 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 <i>et al.</i> 1991 (Finland)	Reinforced-plastics industry workers from 32 workshops (NR)	<i>Past exp. (index pts)</i> <i>Laminators</i> low 12 high 13 <i>Other workers</i> low 10 high 9 <i>Controls</i> other factory 19 plastics factory 12 <i>All subjects</i> exposed 70 controls 31	43 (5–182) 11 (1–133)	(mM) 2.2 (NR) ^c	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> <i>F = 4.66, P = 0.016 for male laminators who smoked compared to other male smoking workers. No overall association with</i>	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	
						<i>styrene exposure</i>	
Yager <i>et al.</i> 1993 (United States)	Boat manufacturing workers (6.4 yr)	<i>Exposed</i> total high medium low	46 11 20 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> <i>Air:</i> <i>r = 0.4, P < 0.01</i> <i>Breath:</i> <i>r = 0.5, P = 0.001</i>	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 <i>et al.</i> 1994 (Germany)	Reinforced- plastics workers (8 yr)	<i>Total</i> laminators laminators formers controls <i>Smokers</i> laminators formers controls <i>Nonsmokers</i> laminators	14 9 ^h 14 20 4 13 10 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 <i>et al.</i> 1994 (Germany)	Polyester resin/fiberglass plastic products production workers (4–31 yr)	<i>Exposed</i> total group 1 group 2 <i>Controls</i>	46 24 22 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> <i>No correlation with exposure duration</i>	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 <i>et al.</i> 1994 (Belgium)	Fiberglass- reinforced- plastics workers (2.9 yr)	<i>Smokers</i> exposed controls <i>Nonsmokers</i> exposed controls	30 9 13 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> <i>No correlation with styrene air levels or</i>	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	
					<i>urinary metabolite levels</i>	<p>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)</p> <p>Relatively low exposure</p> <p>Smoking significantly increased SCE frequency; no correlation of SCE with age, medical history, or other lifestyle factors</p> <p>No association between styrene exposure and HFC was observed.</p> <p>Statistics: two-tailed Mann-Whitney U test</p>
Artuso <i>et al.</i> 1995 (Italy)	Fiber-reinforced-plastics boat building workers (NR)	<p><i>Lab 1</i></p> <p>low 13 high 19 controls 21</p> <p><i>Lab 2</i></p> <p>low 9 high 4 controls 13</p>	<p>NR [0.5–28] NR [20–320]</p> <p>NR [0.5–28] NR [20–320]</p>	NR	<p>2.82 ± 0.12*^f 3.01 ± 0.12* 2.38 ± 0.10</p> <p>6.47 ± 0.50 7.32 ± 0.81 5.44 ± 0.26</p> <p><u>Exposure response</u></p> <p><i>Tests for linear trend significant for both labs</i></p> <p><i>Multiple regression (RR, 95% CI):</i></p> <p><i>Low: 1.22 (1.05–1.43)</i></p>	<p>All subjects were males; controls matched for age and smoking and from the same area as exposed</p> <p>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</p> <p>3 scorers from 2 labs used</p> <p>No significant association with smoking, alcohol consumption and diagnostic X-rays</p> <p>Statistics: <i>t</i>-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</p>

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 <i>et al.</i> 1995 (Germany)	Styrene production plant workers (1–34 yr)	<i>Total</i> Exposed 25 Controls 25 <i>Smokers</i> Exposed 17 controls 13 <i>Nonsmokers</i> 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 <i>t</i> -test
Rappaport <i>et al.</i> 1996 (United States)	Reinforced plastic boat manufacturing workers (≥ 1 yr)	<i>Exposed</i> 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: <i>r</i> = 0.39, <i>P</i> < 0.1 SO– all: <i>r</i> = 0.23, NS SO– smokers: <i>r</i> = 0.81, <i>P</i> = 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 <i>et al.</i> 1997 (Turkey)	Furniture workers (10 yr)	<i>Total</i> exposed 44 controls 41	30 (20–300)	207 (14–1,482) ^c 12 (0–38) ^c	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	
		<i>Smokers</i> exposed 29 controls 29 <i>Nonsmokers</i> exposed 15 controls 12			6.8 ± 1.3 5.88 ± 0.76 5.2 ± 1.4** 3.66 ± 0.52 <u>Exposure response</u> 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 <i>et al.</i> 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 <i>t</i> -test
Laffon <i>et al.</i> 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 <u>Exposure response</u>	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)				<i>Positive correlation with exposure duration (P ≤ 0.01)</i>	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 <i>t</i> -test, one-way ANOVA
Teixeira <i>et al.</i> 2004 (NR)	Reinforced-plastics workers from 2 small plants (1–30) yr	<i>Total</i> exposed 28 controls 28 <i>Men</i> exposed 18 controls 18 <i>Women</i> 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: <i>t</i> -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/m³ to ppm (1 mg/m³ styrene \approx 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 \pm 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.

1 The general limitations [i.e., small numbers of subjects and failure to control for potential
2 confounding factors] noted for chromosomal aberration and micronucleus studies also
3 apply to SCE studies. Most of the studies included control groups matched on one or
4 more of the following variables: age, gender, or smoking. Some studies that did not use
5 matched subjects controlled for variables (such as age, gender, and smoking habits) in the
6 analysis or reported that age, smoking, and gender distribution were similar between
7 groups. Only two studies (Sorsa *et al.* 1991) [it was not clear whether smoking and age
8 were controlled for in the dose-response regression analysis], and Biró *et al.* (2002) did
9 not meet those criteria. Biro *et al.* did report that ages were similar, but smoking habits
10 and gender differed between the exposed and referent groups. Ten studies reported that
11 smoking was significantly correlated with SCE (Hallier *et al.* 1994, Karakaya *et al.* 1997,
12 Kelsey *et al.* 1990, Laffon *et al.* 2002a, Maki-Paakkanen *et al.* 1991, Sorsa *et al.* 1991,
13 Tates *et al.* 1994, Van Hummelen *et al.* 1994, Watanabe *et al.* 1983, Yager *et al.* 1993),
14 while Meretoja *et al.* (1978a), Camurri *et al.* (1983, 1984), Hansteen *et al.* (1984),
15 Brenner *et al.* (1991), Artuso *et al.* (1995), Holz *et al.* (1995), Rappaport *et al.* (1996),
16 and Teixeira *et al.* (2004) did not find an association with smoking. Age was associated
17 with SCE in the study by Sorsa *et al.* (1991) but not in another study (Van Hummelen *et*
18 *al.* 1994). None of the studies reported that gender was a significant factor.

19 Two studies (Rappaport *et al.* 1996, Yager *et al.* 1993) had longitudinal study designs
20 (without controls), and compared styrene exposure measured at several times during a
21 one-year period with SCE frequency. Both studies reported positive correlations with
22 styrene exposure (levels in exhaled air and/or other biomarkers of styrene exposure).

23 Nine of the remaining 20 studies reviewed reported a significant increase in SCEs in
24 workers exposed to styrene compared with controls (Table 5-15). These nine studies
25 included Andersson *et al.* (1980), Camurri *et al.* (1983, 1984) [considered as one study],
26 Hallier *et al.* (1994), Tates *et al.* (1994), Artuso *et al.* (1995), Karakaya *et al.* (1997), Biro
27 *et al.* (2002), Laffon *et al.* (2002a), and Teixeira *et al.* (2004). In addition to the exposure-
28 response relationships observed in the longitudinal studies, Artuso *et al.* (1995) (exposure
29 level), Laffon *et al.* (2002a) (exposure duration), and Watanabe *et al.* (1983) (total
30 urinary metabolites) also reported significant exposure-response associations. However,

1 no associations were reported in other studies (Kelsey *et al.* 1990, Sorsa *et al.* 1991, Tates
2 *et al.* 1994 [for exposure duration], Van Hummelen *et al.* 1994, and Karakaya *et al.*
3 1997). Workers in the study by Tates *et al.* (1994) were exposed to dichloromethane;
4 however, no correlation was found between dichloromethane exposure and SCE levels.
5 Workers in other studies (Camurri *et al.* 1983, 1984, and Laffon *et al.* 2002a) were
6 exposed to other chemicals such as organic peroxides, dyes, and acetone. Andersson *et*
7 *al.* (1980) did not report results for all subjects and reported exposure as the product of
8 styrene concentration and years exposed. Hallier *et al.* (1994) found that SCE levels in
9 laminators decreased after technical and hygienic improvements reduced styrene levels
10 from 37 to 15 ppm. Biró *et al.* (2002) did not provide styrene concentrations or levels of
11 styrene metabolites in the urine.

12 SCE levels were not significantly higher in exposed workers than controls in the studies
13 by Meretoja *et al.* (1978a), Watanabe *et al.* (1983, 1981), Hansteen *et al.* (1984), Mäki-
14 Paakkanen *et al.* (1991), Mäki-Paakkanen (1987), Kelsey *et al.* (1990), Brenner *et al.*
15 (1991), Sorsa *et al.* (1991), Van Hummelen *et al.* (1994), and Holz *et al.* (1995). There
16 were no clear differences in the number of subjects or mean styrene concentrations
17 between the positive and negative studies. However, most of the studies published prior
18 to 1994 were negative while most of the studies published after 1994 were positive. The
19 meta-analysis by Bonassi *et al.* (1996) was inconclusive regarding styrene exposure and
20 SCE.

21 **5.4.5 Genetic polymorphisms and susceptibility to styrene-mediated genotoxicity**
22 Individuals may vary in their susceptibility to styrene's genotoxic effects because of
23 differences in the ability to activate and inactivate styrene or differences in DNA-repair
24 capacity. *CYP2E1* is one of the primary enzymes involved in the metabolism of styrene
25 to styrene-7,8-oxide, and detoxification is mediated by mEH [also known as EPHX1] and
26 glutathione S-transferases (GSTs); conjugation of styrene with glutathione as a minor
27 detoxification pathway (see Section 5.1.3 for a detailed description of the metabolism of
28 styrene). Studies have been conducted *in vitro* with human lymphocytes exposed to
29 styrene and in styrene-exposed workers to evaluate whether polymorphisms in xenobiotic

1 metabolizing enzymes, DNA repair, or other critical pathways modulate genotoxic
2 damage.

3 5.4.5.1 *In vitro studies*

4 The findings of the *in vitro* studies are summarized in Table 5-16. Human lymphocytes
5 with polymorphisms in metabolic enzymes (coded for by *CYP1A1*, *CYP2E1*, *GSTM1*,
6 *GSTT1*, *GSTP1*, or *mEH*) or DNA-repair enzymes (coded for by *hOGG1*, *XRCC1*, or
7 *XRCC3*) were exposed to either styrene (usually at concentrations between 5,000 and
8 10,000 μM , although one study used 500 to 1,500 μM) or styrene-7,8-oxide (usually at
9 10 to 300 μM , although one study used 600 to 2,500 μM). Genotoxicity (single-strand
10 breaks, *HPRT* mutations, micronuclei, or SCE) was measured and compared among the
11 genotypes. [Most studies used only a small number of cells per genotype group. It is
12 difficult to draw any conclusions about the effects of specific polymorphisms in
13 modifying specific damage, because some of the polymorphisms were evaluated in only
14 one study, or conflicting results were observed when the polymorphism was evaluated in
15 several studies (e.g., *GSTM1* and *GSTT1*). Interpretation of the *GSTM1* and *GSTT1*
16 studies also is complicated because the studies looked at different end points (e.g., SCE,
17 *HPRT* mutations) or used different exposure agents (styrene or styrene-7,8-oxide), and
18 some studies looked at combinations of *GSTM1* and *GSTT1* genotypes.] Three studies
19 reported higher levels of genetic damage in a *GSTP1* variant; [however, the studies varied
20 in the exposure (styrene or styrene-7,8-oxide), the end point affected (single-strand
21 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			
<i>CYP1A1</i> Msp I (m1, m2, m4) ^a	var. 4–10 wt 20–26	5,000–10,000	–	SB	more SSB in <i>CYP1A1</i> m1 and m2 heterozygotes; less damage in m4 heterozygotes	Laffon <i>et al.</i> 2003a
<i>CYP2E1</i> RsaI and DraI ^b	var. 1–6 wt 24–29	5,000–10,000	–	SB	more SSB in <i>CYP2E1</i> DraI heterozygotes	Laffon <i>et al.</i> 2003a
<i>EPHX1</i> low, medium, high mEH activity	6–8	–	10–100	SB	no genotype effect	Buschini <i>et al.</i> 2003
	5–16	5,000–10,000	–	SB	no genotype effect	Laffon <i>et al.</i> 2003a
	6–18	–	50–200	MN, SB	more MN and SSB in cells with lower mEH activity	Laffon <i>et al.</i> 2003b
	4–9	–	100–300	MN, SB	more MN in cells with higher mEH activity at 200 μM	Godderis <i>et al.</i> 2006
<i>GSTM1</i> null	6+/6–	–	50–150	SCE	no genotype effect	Uuskula <i>et al.</i> 1995
	3+/2– cell lines ^c	–	600–2,500	HPRT mutation	more mutations in <i>GSTM1</i> -deficient cell lines	Shield and Sanderson 2004, 2001)
	5+/9–	–	10–100	SB	no genotype effect	Buschini <i>et al.</i> 2003
	15+/12–	5,000–10,000	–	SB	no genotype effect	Laffon <i>et al.</i> 2003a
	17+/13–	–	50–200	MN, SB	no genotype effect	Laffon <i>et al.</i> 2003b
	8+/12–	–	100–300	MN, SB	no genotype effect	Godderis <i>et al.</i> 2006
<i>GSTT1</i> null	5+/5–	–	50–150	SCE	more SCE in <i>GSTT1</i> null	Ollikainen <i>et al.</i> 1998
	21+/6–	5,000–10,000	–	SB	no genotype effect	Laffon <i>et al.</i> 2003a
	24+/6–	–	50–200	MN, SB	no genotype effect	Laffon <i>et al.</i> 2003b
	11+/3–	–	10–100	SB	no genotype effect	Buschini <i>et al.</i> 2003
	14+/6–	–	100–300	MN, SB	more MN with <i>GSTT1</i> (considered a spurious effect)	Godderis <i>et al.</i> 2006

Genotypes Polymorphisms	Number of donors	Exposure (μM)		End points	Results (compared with + or wild type)	Reference
		Styrene	Styrene- 7,8-oxide			
<i>GSTM1</i> and <i>GSTT1</i> combinations	5–7	500–1,500	–	SCE	more SCE in <i>GSTM1</i> null/ <i>GSTT1</i> null at high styrene level	Bernardini <i>et al.</i> 2002
<i>GSTP1</i> codon 105 alone or in combination with codon 114 ^d	7 wt/7 var.	–	10–100	SB	more SSB in <i>GSTP1</i> variants	Buschini <i>et al.</i> 2003
	4–18	5,000–10,000	–	SB	more SSB in <i>GSTP1</i> 105 variant	Laffon <i>et al.</i> 2003a
	4–18	–	50–200	MN, SB	more MN (nonsignificant) in <i>GSTP1</i> 105 and 114 variant (combination)	Laffon <i>et al.</i> 2003b
	3–10	–	100–300	MN, SB	no genotype effect	Godderis <i>et al.</i> 2006
<i>hOGG1</i> codon 326 ^e	7–13	–	100–300	MN, SB	no genotype effect	Godderis <i>et al.</i> 2006
<i>XRCC1</i> codons 194 ^f , 280 ^e , 399 ^e	1–18	–	100–300	MN, SB	no genotype effect	
<i>XRCC3</i> 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.

1 5.4.5.2 *In vivo studies*

2 The findings for studies that evaluated polymorphisms in metabolizing enzymes and
3 genotoxic effects in styrene-exposed workers are summarized in Table 5-17. Results
4 evaluating the relationship between polymorphisms and genetic damage in both non-
5 exposed and styrene-exposed workers are not discussed in this review. [Most of these
6 studies determined the genotypes of fewer than 50 styrene-exposed workers for several
7 polymorphisms, measured various genotoxic end points, and compared the amount of
8 genetic damage among genotype groups. As with the *in vitro* studies, it is difficult to
9 draw any conclusions regarding specific polymorphisms, because the findings were
10 conflicting, and the studies had many limitations. The number of individuals per
11 genotype was very small, limiting the statistical power to detect an effect. Many studies
12 did not adjust for potential confounders and made multiple comparisons, thus increasing
13 the possibility of obtaining false-positive results.]

14 One study of styrene-exposed workers evaluated polymorphisms in DNA-repair genes
15 and genotoxicity; however, this study included both styrene-exposed workers and
16 referents, and most of the analysis was of the whole population rather than specifically
17 the styrene-exposed populations. Kuricova *et al.* (2005) measured DNA adducts, single-
18 strand breaks, *HPRT* mutations, and chromosomal aberrations among 48 workers (16
19 males and 32 females) at a styrene-reinforced-plastics plant (the same population studied
20 by Vodicka *et al.* (2001a); see Table 5-17). Levels of damage were compared among
21 polymorphisms for XPD, XPG, XPC, XRCC1, XRCC3, and cyclin D1. Most of the
22 results were presented for the entire population (which also included 24 controls), but the
23 authors stated that styrene-exposed individuals with the *XRCC1* 399 wild-type genotype
24 had a lower frequency of chromosomal aberrations than individuals with the variant
25 genotype. [This was the only significant finding among the exposed population.]

26 [In addition to the limitations discussed (small numbers of subjects or cell lines, multiple
27 comparisons, potential confounding, conflicting results, and very few studies evaluating
28 similar end points and genotypes), the genetic susceptibility studies discussed evaluated
29 only single polymorphisms. The evaluation of multiple loci or several polymorphisms in

- 1 the same pathway may be more informative for identifying populations sensitive to
- 2 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
<i>CYP1A1</i> MspI	44 (19 <i>HPRT</i> 29 SSB)	SSB, CA, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2001a
<i>CYP2E1</i> RsaI and DraI	44 (19 <i>HPRT</i> 29 SSB)	SSB, CA, <i>HPRT</i> mutation	more <i>HPRT</i> mutations in DraI heterozygotes; more SSB in both RsaI and DraI heterozygotes; no effects for CA.	Vodicka <i>et al.</i> 2001a
	19	DNA adducts, <i>HPRT</i>	more adducts in heterozygotes, no effect on adducts	Vodicka <i>et al.</i> 2003
	28	SCE, MN	no effects reported	Teixeira <i>et al.</i> 2004
<i>EPHX1</i> low, medium, high mEH activity	44 (19 <i>HPRT</i> 29 SSB)	SSB, CA, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2001a
	48	SSB	no effects reported	Buschini <i>et al.</i> 2003
	19	DNA adducts, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2003
	28	SCE, MN	decreased SCE for medium mEH activity; no effect on MN	Teixeira <i>et al.</i> 2004
<i>GSTM1</i> null	44 (19 <i>HPRT</i> 29 SSB)	SSB, CA, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2001a
	14	SSB, SCE, MN	no effects reported, but increased PRI ^c was observed in <i>GSTM1</i> null	Laffon <i>et al.</i> 2002a
	48	SSB	fewer SSB in <i>GSTM1</i> null	Buschini <i>et al.</i> 2003
	19	DNA adducts, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2003
	28	SCE, MN	no effects reported	Teixeira <i>et al.</i> 2004
	95	MN, CA	no effects reported	Migliore <i>et al.</i> 2006b
<i>GSTT1</i> null	44 (19 <i>HPRT</i> 29 SSB)	SSB, CA, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2001a
	14	SSB, SCE, MN	no effects reported	Laffon <i>et al.</i> 2002a
	48	SSB	more SSB in <i>GSTT1</i> null	Buschini <i>et al.</i> 2003
	19	DNA adducts, <i>HPRT</i> mutation	no effects reported	Vodicka <i>et al.</i> 2003
	28	SCE, MN	no effects reported	Teixeira <i>et al.</i> 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 <i>et al.</i> 2006b
<i>GSTP1</i> codon 105 alone or in combination with codon 114	44 (19 <i>HPRT</i> 29 SSB)	SSB, CA, <i>HPRT</i>	marginal effect on <i>HPRT</i> mutation ^d no effects reported for SSB and CA	Vodicka <i>et al.</i> 2001a
	19	DNA adducts, <i>HPRT</i> mutation	more <i>HPRT</i> mutations in heterozygotes, no effect on adducts	Vodicka <i>et al.</i> 2003
	28	SCE, MN	no effects reported	Teixeira <i>et al.</i> 2004
	95	MN, CA	no effects reported	Migliore <i>et al.</i> 2006b

^aCA = chromosomal aberrations, MN = micronuclei, SSB = single-strand breaks.

^bResults 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.

^cPRI = 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.

^dThe effect was observed only when outliers were included.

1 **5.4.6 Summary of styrene and styrene-7,8-oxide genotoxicity**

2 Results from *in vitro* studies and *in vivo* studies in experimental animals and humans are
3 summarized in Table 5-18. DNA adducts (primarily O⁶-deoxyguanosine and N7-
4 deoxyguanosine) have been detected in the liver and lungs of rats and mice exposed to
5 styrene by inhalation or i.p. injection. O⁶-deoxyguanosine, N²-guanine, and N1-adenine
6 adducts have been detected in lymphocytes of workers occupationally exposed to styrene.
7 *In vitro* and *in vivo* studies indicated that styrene could induce DNA damage including
8 single-strand breaks. Mutation studies in bacteria were mostly negative without metabolic
9 activation, but some studies were positive with metabolic activation. *In vitro* mutation
10 studies with eukaryotic cells gave mixed results. No mutation studies of styrene-exposed
11 experimental animals were reviewed. A few studies investigated *HPRT*- and *GPA*-locus
12 mutations in styrene-exposed workers and reported inconclusive to weak positive results.
13 One study was positive for *HPRT* mutations but these workers also were exposed to
14 dichloromethane. *In vitro* studies indicate that styrene can cause chromosomal
15 aberrations, SCE, and micronuclei; whereas, *in vivo* studies in rodents were positive for
16 SCE only. A meta-analysis of studies of occupational exposed workers reported a
17 positive association between styrene exposure level (higher levels) and chromosomal

- 1 aberration frequency. Studies in occupationally exposed workers show conflicting
- 2 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.

1 **5.5 Mechanistic studies and considerations**

2 Several recent publications reviewed the possible mechanisms of styrene-induced
3 carcinogenicity. Both genotoxic and epigenetic processes have been considered. IARC
4 (2002) proposed two likely mechanisms for styrene carcinogenicity: (1) DNA damage in
5 target tissues resulting from metabolic conversion of styrene to styrene-7,8-oxide and (2)
6 the cytotoxic effects of styrene in the lungs of mice. IARC did not consider these
7 mechanisms to be mutually exclusive and suggested that the interspecies differences in
8 the metabolism of styrene and styrene-7,8-oxide in rats and mice were likely important.
9 In addition, Cruzan *et al.* (2002) and The Harvard Center for Risk Analysis (Cohen *et al.*
10 2002) concluded that cytotoxicity and subsequent hyperplasia of lung cells must play a
11 key role underlying development of lung tumors in mice, and they proposed several
12 potential mechanisms that could explain how styrene exposure could cause development
13 of hyperplasia in the mouse lung but not the rat lung. Cohen *et al.* (2002) proposed that
14 the species differences between mice and rats (assuming that the lung tumors are caused
15 by styrene-7,8-oxide) could be due to a combination of higher rates of styrene-7,8-oxide
16 accumulation and greater susceptibility of the mouse lung to epoxides. IARC (2002)
17 noted that mice are considered to be more susceptible to induction of lung tumors by
18 epoxides and chemicals capable of being metabolized to epoxides than are rats, based on
19 findings of lung tumors in mice but not in rats when both species were exposed to
20 ethylene oxide, 1,3-butadiene, isoprene, or chloroprene. Cruzan *et al.* (2002) proposed
21 that interspecies differences in styrene toxicity are most likely explained through CYP2F-
22 generated metabolites such as 4-vinylphenol.

23 Some (but not all) studies in experimental animals reported increased incidences of
24 tumors of the mammary gland or lymphatic system in rats (see Section 4), and increases
25 in mortality or incidence of pancreatic cancer and lymphohematopoietic malignancies
26 have been reported in some studies of styrene-exposed workers (see Section 3). However,
27 no styrene-specific mechanistic studies or reviews of the mammary gland, pancreas, or
28 lymphohematopoietic system as possible tumor sites were identified. (See Section 5.2 for
29 a discussion of prolactin, styrene exposure, and breast cancer). The following sections
30 discuss mechanistic considerations related to genotoxicity (Section 5.5.1), gene
31 expression and apoptosis (Section 5.5.2), oxidative stress (Section 5.5.3), cytotoxic

1 effects of styrene on mouse lung (Section 5.5.4), and selected styrene analogues (Section
2 5.5.5).

3 Styrene-7,8-oxide, a primary and genotoxic metabolite of styrene, is listed in the *Report*
4 *on Carcinogens* as *reasonably anticipated to be a human carcinogen* based on sufficient
5 evidence in experimental animals (NTP 2004), and IARC (1994b) concluded that there
6 was sufficient evidence in experimental animals for its carcinogenicity. Styrene-7,8-oxide
7 and other epoxides, or epoxide-forming chemicals, are reactive compounds. Epoxides
8 have been associated with lung, liver, harderian gland, and circulatory system neoplasms
9 in mice; Zymbal's gland and brain tumors in rats; and mammary gland and forestomach
10 tumors in rats and mice (Melnick 2002). Dunnick *et al.* (1995) and Bennett and Davis
11 (2002) reviewed findings from NTP's carcinogenesis studies and reported that epoxides
12 or chemicals metabolized to epoxides were associated with mammary tumors in rodents.
13 Bennett and Davis hypothesized that the mammary gland may be efficient in
14 metabolizing chemicals to their epoxides. However, these authors also noted that not all
15 epoxides or epoxide-forming chemicals were associated with mammary tumors. Styrene-
16 7,8-oxide administered by oral gavage induced high incidences of both benign and
17 malignant tumors of the forestomach in both sexes of rats (three strains tested) and in one
18 strain of mice (IARC 1994b). One of the rat studies also included prenatal exposure
19 followed by postnatal gastric lavage. Lijinsky (1986) also reported liver tumors in male
20 mice in the low-dose group only. Lung and mammary tumors were not increased in these
21 studies. No inhalation carcinogenicity studies have been conducted with styrene-7,8-
22 oxide. However, styrene-7,8-oxide has been measured in the blood of rats and mice
23 following oral and i.p. administration (IARC 1994b). No reports of the levels of styrene-
24 7,8-oxide in the lungs of rats or mice exposed to styrene were identified, but a PBPK
25 model indicated that oral administration of styrene-7,8-oxide at 275 mg/kg per day would
26 result in higher lung levels of styrene-7,8-oxide than from metabolism of styrene
27 administered at 40 ppm by inhalation (Sarangapani *et al.* 2002) (see Section 5.3.4 for the
28 metabolic assumptions for this model).

29 An increased incidence of lung, liver, mammary gland, and lymphatic neoplasias has
30 been reported for some studies in experimental animals (see Section 4), although the

1 results were not consistent across studies. Increases in mortality or incidence of
2 lymphohematopoietic malignancies and tumors at some other sites (such as the pancreas)
3 have also been reported in some studies of styrene-exposed workers (see Section 3).
4 However, no styrene-specific mechanistic studies or reviews of the mammary gland,
5 pancreas, or lymphohematopoietic system as possible tumor sites were identified. (See
6 Section 5.2 for a discussion of prolactin, styrene exposure, and breast cancer). The
7 following section discusses mechanistic considerations related to genotoxicity, gene
8 expression and apoptosis, oxidative stress, cytotoxicity in mouse lung, and studies of
9 selected styrene analogues.

10 5.5.1 Genotoxicity

11 Some DNA adducts are highly xenobiotic-specific DNA lesions that can alter DNA
12 ultrastructure. IARC (2002) noted that a potential mechanism for the carcinogenicity of
13 styrene is based on covalent binding of the DNA-reactive metabolite styrene-7,8-oxide.
14 DNA adducts formed with styrene-7,8-oxide include N7-guanine, N3-adenine, O⁶-
15 guanine, N²-guanine, N1-adenine, N⁶-adenine, and N3-cytosine (see Section 5.4.1).
16 Adducts associated with oxidative damage (e.g., 8-hydroxy-2'-deoxyguanosine) also have
17 been reported in styrene-exposed workers (Marczynski *et al.* 1997a). N7-guanine adducts
18 are the predominant type, but are repaired *in vivo*, whereas O⁶-guanine adducts occur at a
19 much lower frequency but are more persistent (see Section 5.4.2.1). N7-guanine and N3-
20 adenine adducts may result in depurination or may cause single-strand breaks. Because
21 DNA polymerase preferentially adds an adenine opposite an apurinic site, N7-guanine
22 adducts may result in G·C→A·T transitions, and N3-adenine adducts may result in
23 A·T→T·A transversions (Loeb and Preston 1986). The other adducts occur at base-
24 pairing sites and may cause the following specific base-pair mutations: (1) O⁶-guanine,
25 G·C→A·T transition, (2) N2-guanine, G·C→A·T transversion (via incorporation of
26 deoxythymidine triphosphate opposite the adduct) (Zang *et al.* 2005b), (3) N1-adenine,
27 mutations at A·T base pairs (via blockage of a central hydrogen bonding site of the
28 adenine residue), (4) N3-uracil, G·C→A·T transition and, to a minor extent, G·C→T·A
29 transversion (via conversion to the N3-cytosine adduct) (Zhang *et al.* 1995), and (5) N6-
30 guanine, A·T→G·C transition. A·T→G·C transition was the dominant type of mutation in

1 both styrene-7,8-oxide–exposed *HPRT* mutant clones (Bastlová and Podlutzky 1996) and
2 in a site-specific mutation study in which a styrene-7,8-oxide adduct at the N⁶-position of
3 adenine was inserted at codon 61 in the *N-ras* gene (Latham *et al.* 1993). Weak
4 mutagenicity was observed when *S*-styrene-7,8-oxide was bound at the α -carbon of
5 styrene-7,8-oxide to the adenine in the second position of the codon, while the *R*-
6 enantiomer bound at that position blocked replication of the single-stranded DNA
7 template almost completely, and no mutagenicity was found when either the *R*- or the *S*-
8 enantiomer was bound to the adenine in the third position of codon 61. The β -N6-dA
9 styrene-7,8-oxide adducts have been examined as to site-specific mutagenesis in *E. coli*.
10 These data indicate that the β -N6-dA adducts do not have significant deleterious effects
11 on replication competence (Kanuri *et al.* 2001).

12 The actions of native and various site-specific mutants of HIV-1 reverse transcriptase
13 have been examined *in vitro* on DNA templates modified with α -N6-dA adducts. For the
14 native enzyme, activity is dependent on both the chirality of the N6-dA adducts and their
15 sequence contexts. Replication is possible but is terminated 3 to 5 nucleotides after
16 translesion synthesis and before reaching the end of the template (Latham and Lloyd
17 1994). Eight mutants of reverse transcriptase also terminate synthesis on these styrene-
18 7,8-oxide–adducted templates. The sites of termination occur primarily 1 and 3 bases
19 following adduct bypass, when the lesion is positioned in the major groove of the
20 template-primer stem (Latham *et al.* 2000).

21 Similar replication assays have been performed using *E. coli* Klenow fragment,
22 Sequenase 2.0, T4 polymerase holoenzyme, polymerase α , and polymerase β , *in vitro*. In
23 all instances, lesion bypass is sensitive to both the local sequence context and the
24 chirality of the α -N6-dA styrene-7,8-oxide adducts. For example, in the 5'-AXG-3'
25 sequence, adducts having *R*-stereochemistry are bypassed, whereas stereochemically-
26 identical lesions in other sequence contexts are often poor substrates. Similarly, *R*- vs. *S*-
27 α -N6-dA adducts introduced within identical sequences are often bypassed
28 nonequivalently. The degree of adduct-directed termination and translesion synthesis
29 during replication is also dependent on the choice of polymerase. Templates that are poor
30 substrates for bypass synthesis with one enzyme often read through much more

1 efficiently when a different polymerase is used (Latham *et al.* 1995). Similar studies have
2 been conducted using reconstituted *E. coli* Pol III. Replication is poorly processive and
3 strongly terminated by styrene-7,8-oxide lesions in 33-mer templates, although the same
4 enzyme showed efficient bypass of the same adducts in M13 DNA (Latham *et al.* 1996).
5 No data are available regarding replication by Y-family polymerases, *in vitro*.

6 Single-strand breaks were observed in most studies of styrene-exposed workers and
7 occurred in a concentration-dependent manner (see Section 5.4.4.2). Most studies have
8 reported an increase in chromosomal aberrations in styrene-exposed workers, and
9 exposure-response relationships have been observed in several studies. A meta-analysis
10 (Bonassi *et al.* (1996) of studies published prior to 1996 found a positive association
11 between styrene exposure (greater than 30 ppm for an 8-hour time-weighted average) and
12 chromosomal aberrations. The data on mutations and other types of genetic damage in
13 humans are conflicting. Most recent studies have reported higher levels of sister
14 chromatid exchange in styrene-exposed workers than in controls, but the study
15 populations were small, and potential confounding was not always addressed.

16 As described in Section 4, styrene exposure caused lung tumors in mice but not in rats. *In*
17 *vivo* experiments in rodents have shown that styrene exposure can cause DNA adducts in
18 lung and liver in mice and rats (see Section 5.4.3.1 and Table 5-7 for a description of
19 these studies). Comparison between animal studies is difficult because different species,
20 organs, methods of detection, routes of administration, and exposure levels were used.
21 Moreover, most genotoxicity studies in animals are short-term, and humans are exposed
22 for long time periods. No correlation of adducts with tumor incidence has been observed
23 (Nestmann *et al.* 2005), suggesting that other mechanisms of carcinogenicity may also be
24 important. Boogaard *et al.* (2000b) reported that styrene had a low covalent binding index
25 (CBI) relative to other known genotoxicants; the hepatic CBI (at 42 hours) was 0.19 in
26 rats and 0.44 in mice, and the pulmonary CBI (pooled 0 and 42 hours) was 0.17 in rats
27 and 0.24 in mice. The low levels of styrene-7,8-oxide adducts in the forestomach as the
28 target tissue for styrene-7,8-oxide in rats and mice were judged to be insufficient to
29 account for its carcinogenic activity by a strictly genotoxic mechanism (Phillips and
30 Farmer 1994) (see Section 5.4.3.1). However, the reported levels of DNA binding varied

1 by factors of 20 to 50 among the studies. The reasons for the discrepancies were not
2 completely understood, according to the authors; however, some of the variability could
3 be attributed to differences in administration routes, measurement methods, and losses
4 through depurination.

5 Koskinen *et al.* (2001a) compared the formation of β -N1-adenine adducts resulting from
6 styrene exposure in mice and humans. They reported that exposure of mice to styrene at
7 750 mg/m^3 [173 ppm] resulted in formation of 1 β -N1-adenine adduct per 10^9 normal
8 nucleotides, while exposure of humans at 76 mg/m^3 [17.5 ppm] resulted in 0.8 adducts
9 per 10^9 nucleotides [the results are reported in Table 5-10 as 0.08 adducts per 10^8
10 nucleotides].

11 5.5.2 Gene expression and apoptosis

12 The effect of styrene-7,8-oxide on the expression of genes involved in the cell cycle and
13 in regulation of apoptosis was studied in white blood cells exposed to styrene-7,8-oxide
14 at a concentration of 50 or 200 μM (Laffon *et al.* 2001a). mRNA and reverse
15 transcription polymerase chain reaction were used to analyze the expression of the genes
16 involved in cell-cycle arrest in response to DNA damage (*p53*, *p21*) or in control of
17 apoptosis (*bcl-2* and *bax*). Apoptotic events were detected by the DNA fragmentation
18 assay. Data for expression were presented only in the form of graphs for individual
19 donors (2 men and 2 women described as healthy nonsmokers aged 23 to 30). The
20 authors reported high interindividual variation in the expression of studied genes, with no
21 consistent pattern of increased or decreased expression. The authors did describe a
22 difference in the cytokinesis block proliferation index (CBPI). All CBPI values for
23 control cultures and low-exposure cultures were between 1.94 and 2.04, while the values
24 for high-exposure cultures ranged from 1.67 to 1.78 and were significantly lower than in
25 the controls ($P < 0.01$), indicating a delay in cell-cycle kinetics. The authors suggested
26 that exposure to high levels of styrene-7,8-oxide might induce a delay in the cell cycle,
27 which could allow the DNA repair system to act on the genotoxic damage produced,
28 instead of driving the cells towards programmed cell death.

1 Diodovich *et al.* (2004) studied the effect of styrene on cell-cycle gene (*c-fos*, *c-jun*)
2 expression profiles in human cord blood cells and styrene's effect on production of
3 apoptosis-related proteins (Bax, Bcl-2, Raf-1). Exposure to styrene at 800 μ M for 24 or
4 48 hours increased necrosis of mononuclear cord blood cells, but not apoptosis. Western
5 blot analysis revealed induction of both c-jun and c-fos protein, but at different times, as
6 c-jun was induced early and decreased later, while c-fos was induced only after 48 hours
7 of exposure to styrene. Production of both Bcl-2 and Raf-1 proteins was induced by
8 styrene exposure at all time points (6, 24, and 48 hours), whereas Bax protein was
9 initially downregulated but recovered at the later times. The p53 protein was not
10 produced in either unexposed or styrene-exposed cells. Macroarray analysis (see
11 Glossary) showed that styrene-modified cord blood gene expression was associated with
12 upregulation of monocyte chemotactic protein and downregulation of CC chemokine
13 receptor type 1 and SLP-76 tyrosine phosphoprotein. The authors concluded that their
14 results supported a role for styrene in promotion of cell proliferation and cell-cycle
15 progression, which could potentially favor alterations in gene expression and genotoxic
16 effects.

17 5.5.3 Oxidative stress

18 Marczynski *et al.* (2000) proposed a mechanism involving oxidative stress and oxidative
19 DNA damage as the basis for the genotoxic effects of styrene resulting from an
20 imbalance between oxidants and antioxidants in cells. Gamer *et al.* (2004) (see Section
21 5.2.3.2) found no evidence of oxidative stress as indicated by unchanged concentrations
22 of 8-OH-deoxyguanosine in lung lavage fluid after 20 daily (6 hours per day during a 4-
23 week period) exposures to styrene at 20, 40, 80, or 160 ppm. However, Roder-Stolinski *et al.*
24 (2008) reported that exposure of human lung epithelial cells (cell line A549) to
25 styrene *in vitro* stimulated the expression of inflammatory mediators, including
26 chemotactic cytokine monocyte chemoattractant protein-1 (MCP-1) through activation of
27 the NF- κ B signaling pathway, and suggested that activation of the NF- κ B signaling
28 pathway was mediated via a redox-sensitive mechanism (see Section 5.2.1). Cohen *et al.*
29 (2002) in their review suggested that the pulmonary hyperplasia that occurs in mice but
30 not in rats likely results from oxidative damage that is caused either directly by styrene
31 oxide or indirectly because of depletion of glutathione (GSH). As mentioned above

1 (Section 5.5.1) adducts associated with oxidative damage have been reported in styrene-
2 exposed workers

3 5.5.4 Cytotoxic effects of styrene on mouse lung

4 This section discusses cytotoxicity of styrene metabolites as a possible mechanism of
5 styrene-induced carcinogenesis. Cytotoxicity and cellular proliferation (see Section
6 5.2.2.2) have been observed, especially in mouse lung Clara cells, following *in vitro* and
7 *in vivo* exposure to styrene and styrene metabolites (styrene-7,8-oxide and 4-
8 vinylphenol). Chronic cytotoxicity can result in clonal expansion of styrene-induced, or
9 spontaneous, mutants. Induction of lung tumors resulting from formation of cytotoxic
10 metabolites has been proposed for other chemicals, including naphthalene. A scientific
11 panel at the Naphthalene State-of-the-Science Symposium on the pathogenesis of
12 respiratory tumor formation in rodents (Bogen *et al.* 2008) hypothesized that nasal
13 tumors in rats and lung adenomas in mice occur through a cytotoxic mechanism.
14 Metabolic activation (via Cyp2f2) was required and mouse Clara cells had the greatest
15 capacity to metabolize naphthalene and were also highly susceptible to naphthalene-
16 induced cytotoxicity.

17 Cohen *et al.* (2002), Cruzan *et al.* (2002), and IARC (2002) proposed that styrene
18 exposure causes pulmonary hyperplasia in the mouse lung, which may play a role in the
19 development of lung tumors. Effects of repeated styrene exposure reported in the lungs of
20 mice, but not in rats, included focal crowding of bronchiolar cells, bronchiolar epithelial
21 hyperplasia, and bronchiolo-alveolar hyperplasia (IARC 2002) (see Section 5.2.2.2 for a
22 description of pneumotoxicity in rodents). Studies by Gadberry *et al.* (see Section
23 5.2.2.3) showed that styrene-7,8-oxide administered by i.p. injection caused pulmonary
24 toxicity in mice, suggesting that styrene-7,8-oxide is responsible for the pneumotoxicity
25 and that systemically available styrene-7,8-oxide can enter the lung cell. Cohen *et al.*
26 postulated that styrene might induce cytotoxicity by directly damaging the cell or by
27 causing glutathione depletion (see Section 5.2.2.4). Tissue damage leads to hyperplasia,
28 which makes the tissue more sensitive to tumor development. Cohen *et al.* (2002) also
29 stated that the role of hyperplasia does not rule out the possibility that styrene-7,8-oxide
30 also causes genotoxic effects.

1 Cohen *et al.* (2002) identified three factors that they considered as possible mechanisms
2 contributing to the development of hyperplasia in mice and subsequent development of
3 lung tumors: (1) the presence of the cytochromes CYP2E1 and Cyp2f2, which convert
4 styrene to styrene-7,8-oxide, in mouse lung tissues, (2) greater formation of the *R*-
5 enantiomer of styrene-7,8-oxide, which the authors considered the more toxic and
6 mutagenic of the two enantiomers, and (3) the susceptibility of mouse lung tissue to GSH
7 depletion, which could reduce the detoxification of styrene-7,8-oxide. After carefully
8 comparing these factors in rats, mice, and humans (see Section 5.3 for a review of the
9 literature on interspecies differences, including Cohen *et al.* 2002), the authors concluded
10 that these measures of metabolic activity and styrene-7,8-oxide accumulation in the lung
11 do not explain why styrene caused lung tumors in mice and not rats. IARC (2002)
12 reached a similar conclusion regarding lung-tumor susceptibility and toxicokinetic
13 differences between mice and rats.

14 Cohen *et al.* (2002) proposed several reasons why styrene-7,8-oxide production in the
15 lung did not explain the differences between mice and rats in development of pulmonary
16 hyperplasia: (1) the Harvard PBPK model did not consistently predict a higher
17 concentration of styrene-7,8-oxide in the lungs of mice than in rats, (2) styrene-7,8-oxide
18 concentrations were lower in the blood of mice than in rats by an order of magnitude, and
19 (3) the predicted concentrations of styrene-7,8-oxide in the lungs were very similar for
20 mice exposed to styrene by inhalation at 40 to 80 ppm, which resulted in lung tumors,
21 and rats exposed at 1,000 ppm, which did not induce lung tumors. Cohen *et al.* also stated
22 that it was not clear that the pulmonary toxicity of the *R*-styrene-7,8-oxide was
23 substantially greater than that of the *S*-styrene-7,8-oxide; differences in toxicity appear to
24 be greater in the liver than in the lung (Gadberry *et al.* 1996, see Section 5.2.4). The
25 authors also did not think that the greater sensitivity of mice to GSH depletion (see
26 Section 5.2.2.4) could explain the differences in lung tumor susceptibility, because
27 styrene caused hyperplasia in mice at concentrations (20 ppm) that do not cause GSH
28 depletion. Cohen *et al.* (2002) proposed the following three possible explanations for the
29 difference in susceptibility: (1) a greater number of Clara cells in mouse pulmonary tissue
30 than in rat pulmonary tissue, (2) a pharmacokinetic difference at the cellular level, and (3)

1 a pharmacodynamic difference, such as greater susceptibility at the cellular level to injury
2 due to less efficient DNA repair.

3 Similar to the first of the three factors put forward by Cohen *et al.* (2002) as possible
4 mechanisms for the development of hyperplasia and lung tumors in mice (see above),
5 Cruzan *et al.* (2002) proposed that interspecies differences in styrene toxicity are most
6 likely explained through CYP2F-generated metabolites (2f2 in mice, 2F4 in rats, and 2F1
7 in humans). They noted that almost all of the effects of cytotoxicity and tumor formation
8 were seen in tissues that are high in CYP2F isoforms and that CYP2F inhibitors
9 prevented cytotoxicity (see Section 5.1.3.5). Metabolites formed from ring oxidation,
10 including 4-vinylphenol, are about 6-fold higher in mice compared with rats, and 4-
11 vinylphenol has been reported to be more potent than styrene-7,8-oxide as a
12 pneumotoxicant (see Section 5.1.3.5). Also, styrene metabolism occurs primarily in Clara
13 cells (see Sections 5.1.3.3 and 5.1.3.5), and mice produce higher levels of toxic
14 metabolites (*R*-styrene-7,8-oxide, 4-vinylphenol, and oxidized reactive intermediates of
15 4-vinylphenol), and have a lower level of detoxifying epoxide hydrolase activity than rats
16 or humans (see Sections 5.1.3.1 and 5.1.3.2). They stated that PBPK models predicted
17 that humans do not generate sufficient levels of these metabolites in the terminal
18 bronchioles to reach toxic levels. Cruzan *et al.* stated that the tumor profile of styrene
19 suggests a non-genotoxic mode of action since they felt that the tumors in animals were
20 common, reported in only one species and one site, did not occur at the 12-month
21 sacrifice, and were associated with organ toxicity and cell turnover. Studies published
22 after Cruzan *et al.*'s 2002 proposal that evaluated the role of Cyp2f2, ring-oxidized
23 metabolites, and cytotoxicity in the lung are discussed in Sections 5.1.3.5 and 5.2.2.2. For
24 example, Kaufmann *et al.* (2005) concluded that the side-chain hydroxylation pathway
25 appeared to be of minor relevance for the pneumotoxic effects of styrene (see Section
26 5.2.2.2).

27 5.5.5 Selected styrene analogues

28 Studies on styrene analogues such ethylbenzene, 1-phenylethanol, 4-methylstyrene, and
29 vinyltoluene (a mixture of 3- and 4-methylstyrene) (see Table 1-4 for structures of these
30 analogues), provide further information on the possible relationship between formation of

1 ring-oxidized metabolites and the development of lung tumors in experimental animals
2 exposed to these molecules. [However, no comprehensive reviews or evaluations of all
3 analogues were identified in the peer-reviewed literature, and thus, only a few analogues
4 are discussed. No long-term carcinogenicity studies (such as studies in *Cyp2f2*-knockout
5 mice) that evaluated this proposed mechanism were identified.]

6 Ethylbenzene is a synthetic precursor for styrene (see Section 2.2) differing from styrene
7 only in the absence of the double bond in the 2-carbon side chain, and 1-phenylethanol is
8 a metabolite of both ethylbenzene and styrene (see Figure 5-1). Midorikawa *et al.*
9 reported that ethylbenzene was metabolized to 1-phenylethanol, 2-ethylphenol, and 4-
10 methylphenol by rat liver microsomes. The latter two metabolites were metabolically
11 transformed to the ring-dihydroxylated metabolites ethylhydroquinone and 4-
12 ethylcatechol (in a separate reaction), respectively, and Midorikawa *et al.* proposed
13 further metabolism of the ethylcatechol to 4-ethyl-1,2-benzoquinone. [No *in vivo*
14 metabolism studies were identified.] Incubation of 4-ethylcatechol with calf thymus DNA
15 *in vitro* resulted in oxidative DNA damage, including the formation of 8-oxo-2'-
16 deoxyguanosine (8-oxodG) in the presence of Cu(II), and the oxidative stress resulting
17 from the formation of reactive oxygen species as a result of this proposed metabolic
18 pathway for ethylbenzene could contribute to the carcinogenic mechanism of
19 ethylbenzene. (Oxidative stress has been proposed to play a role in styrene-induced
20 carcinogenicity — see above.) Ethylbenzene has been reported to induce lung tumors in
21 male mice, liver tumors in female mice, kidney tumors in rats (both sexes), and testicular
22 tumors in rats (Chan *et al.* 1998). Stott *et al.* (2003) reported that chronic exposure to
23 ethylbenzene induced changes in the mouse lung, including multifocal
24 bronchiolar/parabronchiolar hyperplasias and altered tinctorial properties. The authors
25 proposed a nongenotoxic mode of action that was dependent upon cell proliferation and
26 altered cell population dynamics. However, no studies were identified that evaluated the
27 role of the ring-oxidized metabolites in lung tumor formation.

28 Other styrene analogues listed above, i.e., 4-methylstyrene and vinyltoluene, are not
29 predicted to form 4-phenol metabolites because of the placement of the methyl group at
30 the 3- or 4- position in these molecules, and no evidence for induction of mouse lung

1 tumors by either of these molecules was identified. However, the findings for lung
2 cytotoxicity were mixed. 4-Methyl styrene (*para*-methylstyrene) was administered by
3 gavage to Sprague-Dawley rats (0, 10, 50, 250, and 500 mg/kg per day) and Swiss mice
4 (0, 10, 50, and 250 mg/kg per day) in a long-term (104 weeks) carcinogenicity study, and
5 no increased tumor incidence was reported compared with control animals. However,
6 Conti *et al.* (1988) reported data only for tumor incidences and did not report any
7 endpoints for possible lung toxicity. [No inhalation studies were identified.] Vinyltoluene
8 (a mixture of 65% to 71% 3-[*meta*-] isomer and 32% to 35% 4-[*para*-] isomer) was tested
9 in a two-year inhalation study in F344 rats (0, 100, or 300 ppm) and B6C3F₁ mice (0, 10,
10 or 25 ppm), and no increase in tumor incidences were reported (NTP 1990a). However,
11 vinyltoluene did cause focal chronic active inflammation and diffuse hyperplasia of the
12 respiratory epithelium, and chronic active inflammation of the bronchioles.

13 No ring-oxidized metabolites of 1-phenylethanol resulting from metabolism of
14 ethylbenzene by mouse microsomes were identified in the study by Midorikawa *et al.*
15 (2004), and neither lung tumors nor lung cytotoxicity were observed when α -
16 methylbenzyl alcohol (1-phenylethanol) was administered by gavage to F344 rats and
17 B6C3F₁ mice at doses of 0, 375, or 750 mg/kg per day in a two-year bioassay (NTP
18 1990b). However, there was an increased incidence of renal tubular-cell adenoma or
19 adenocarcinoma (combined) in male rats and transitional-cell papillomas of the urinary
20 bladder occurred in two high-dose female rats. [No inhalation studies with 1-
21 phenylethanol were identified.]

22 α -Methylstyrene, another chemical tested in a two-year inhalation study, did not
23 significantly increase the incidence of lung tumors or cause lung cytotoxicity in mice (in
24 the two-year study) although it did cause renal tumors and possibly leukemia in male rats
25 and liver tumors in male (marginal) and female mice (NTP 2007). No metabolism studies
26 evaluating whether a 4-phenol derivative of this molecule is formed during metabolism
27 were identified, but its chemical structure does not appear to make that impossible.

1 **5.6 Summary**

2 *5.6.1 Absorption, distribution, metabolism, and excretion*

3 Styrene can be absorbed through inhalation, ingestion, or skin contact, but the most
4 important route of exposure in humans in occupational settings is by inhalation, which
5 results in rapid absorption and distribution of approximately 60% to 70% of inhaled
6 styrene; the highest tissue concentrations are in subcutaneous fat. Food is also an
7 important source of exposure for the general population. Metabolic activation of styrene
8 results in formation primarily of the genotoxic metabolite styrene-7,8-oxide, which can
9 be detoxified by glutathione conjugation or conversion to styrene glycol by microsomal
10 epoxide hydrolase. Styrene is metabolized in both the liver and the lung, and the Clara
11 cells in the lung are regarded as the major cell type in styrene activation following
12 inhalation exposure. The initial step in styrene metabolism is catalyzed by cytochromes
13 P450; CYP2E1 and Cyp2f2 are the predominant enzymes in humans and experimental
14 animals. In animals, CYP2E1 predominates in liver, while Cyp2f2 is the primary enzyme
15 in mouse lung. CYP2A13, CYP2F1, CYP2S1, CYP3A5, and CYP4B1 are preferentially
16 expressed in the lung compared with liver in humans, and the human CYP2F1 has been
17 shown to be capable of metabolizing styrene when expressed *in vitro*. Because styrene-
18 7,8-oxide contains a chiral carbon, this and some subsequent styrene metabolites can
19 exist as either *R*- or *S*-enantiomers. A second metabolic pathway through styrene-3,4-
20 oxide results in formation of 4-vinylphenol, which has been detected in humans, rats, and
21 mice *in vivo*, but the importance of 4-vinylphenol in styrene toxicity has not been well
22 characterized. Almost all absorbed styrene is excreted as urinary metabolites, primarily
23 mandelic acid and phenylglyoxylic acid.

24 *5.6.2 Toxicity*

25 Styrene exposure has been associated with numerous health effects in humans and
26 laboratory animals. The acute toxicity of styrene is low to moderate with an oral LD₅₀ of
27 320 mg/kg and an inhalation LC₅₀ of 4,940 ppm (4-hour exposure) in mice and an oral
28 LD₅₀ of 5,000 mg/kg and an inhalation LC₅₀ of 2,770 ppm (2-hour exposure) in rats. The
29 primary effects of acute exposure to styrene in experimental animals and humans include
30 irritation of the skin, eyes, and respiratory tract and CNS effects. Drowsiness, listlessness,
31 muscular weakness, and unsteadiness are common signs of systemic styrene intoxication.

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

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

21 *5.6.3 Interspecies differences in metabolism, toxicity, and toxicokinetics*

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

1 5.6.4 Genetic and related effects

2 *In vitro* studies show that styrene-7,8-oxide forms DNA adducts and causes single-strand
3 breaks in a dose-related manner. Several studies have shown a correlation between
4 single-strand breaks and DNA adducts and indicate that the strand breaks, which are not
5 generally regarded as significantly lethal or mutagenic lesions, are efficiently repaired
6 within several hours after exposure has stopped. Adducts are formed primarily at the N7-,
7 N²-, and O⁶-positions of guanine. N7-adducts are formed in the greatest amount but are
8 the least persistent, while O⁶-adducts are formed in the least amount but are the most
9 persistent. Styrene-7,8-oxide was mutagenic without metabolic activation in all *in vitro*
10 mutagenicity test systems reported and caused mutations in some studies in the presence
11 of metabolizing enzymes. Both styrene and styrene-7,8-oxide caused cytogenetic effects
12 (sister chromatid exchange [SCE], chromosomal aberrations, and micronuclei) in human
13 lymphocytes or other mammalian cells *in vitro*. DNA adducts have been detected in liver
14 and lung cells of mice and rats exposed to styrene *in vivo*, although the levels varied
15 across studies. The majority of studies in experimental animals demonstrated an effect of
16 both styrene-7,8-oxide and styrene exposure on single-strand breaks, while both positive
17 and negative results for cytogenetic or clastogenic effects of styrene were reported.

18 DNA adducts, primarily N7- and O⁶-adducts, were reported in white blood cells in all
19 studies of styrene-exposed workers employed mainly in hand-lamination plants. In most
20 studies in workers, single-strand breaks showed exposure-related increases; however, two
21 studies gave negative results. The limited data on mutation frequencies in *HPRT* and
22 *GPA* in styrene-exposed workers are inconclusive. More than half the studies measuring
23 chromosomal aberrations have reported an increase in chromosomal aberrations in
24 styrene-exposed workers (or subgroups of workers), and several studies have reported a
25 positive exposure-response relationship with styrene air levels or urinary metabolites. A
26 meta-analysis of 22 studies found a positive association between styrene exposure level
27 and chromosomal aberration frequency when exposure levels were dichotomized as
28 greater than or less than a threshold value of 30 ppm for an 8-hour time-weighted
29 average. Studies of other cytogenetic markers in humans are conflicting. About half of
30 the studies that evaluated micronucleus and SCE frequency in styrene workers were
31 positive, and a few studies have reported significant dose-response relationships with

1 styrene exposure. A meta-analysis of 10 micronucleus studies was inconclusive, and a
2 meta-analysis of 14 SCE studies indicated a slight increase in SCE frequency but, again,
3 was too small to be conclusive. A number of studies have been published on the possible
4 modulating role of genetic polymorphisms, mainly in xenobiotic metabolism enzymes
5 and DNA-repair genes, at the level of various biomarkers. Some authors have suggested
6 that genetic susceptibility (probably at many loci) may be important in styrene-mediated
7 genotoxicity.

8 5.6.5 Mechanistic studies and considerations

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

24 Many researchers have tried to explain why lung tumors were observed in mice but not in
25 rats in long-term inhalation exposure studies. Some researchers have proposed that
26 styrene exposure causes pulmonary hyperplasia in the mouse lung, which may play a role
27 in the development of lung tumors. Effects of repeated styrene exposure observed in the
28 lungs of mice, but not in rats, included focal crowding of bronchiolar cells, bronchiolar
29 epithelial hyperplasia, and bronchiolo-alveolar hyperplasia. The Harvard Center for Risk
30 Analysis (Cohen *et al.* 2002) considered three factors as possible explanations for the

1 greater susceptibility of mouse lung than rat lung to development of hyperplasia leading
2 to tumors with exposure to styrene are: (1) the presence of the styrene-metabolizing
3 cytochromes in mouse lung tissues, (2) greater formation of the *R*-enantiomer of styrene-
4 7,8-oxide, and (3) the susceptibility of mouse lung tissue to glutathione depletion.
5 However, they concluded that although toxicokinetic models generally predict higher
6 rates of metabolism by mice and rats than by humans, the models do not consistently
7 predict a difference between the rodent species. An alternative mechanism is that
8 interspecies differences in styrene toxicity are most likely explained through CYP2F-
9 generated metabolites (2f2 in mice, 2F4 in rats, and 2F1 in humans) in the mouse lung.
10 This is based on data showing that most of the effects of cytotoxicity and tumor
11 formation were seen in mouse respiratory tissues, which are high in CYP2F isoforms, and
12 that CYP2F inhibitors prevented cytotoxicity. Moreover, metabolites formed from ring
13 oxidation, including 4-vinylphenol, are about 6-fold higher in mice compared with rats,
14 and 4-vinylphenol is more potent than styrene-7,8-oxide as a pneumotoxicant.

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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 surface 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.

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Erratum and Addendum to the Final Report on Carcinogens Background Document for Styrene

The following corrections are made to the Final Report on Carcinogens Background Document for Styrene.

1. Table 3-10, page 187. The footnote * is corrected to read: “Note that Kolstad *et al.* classified employees at companies with 50% or more of workers involved in reinforced plastics as probable high exposure, and that most of the companies were boat yards or manufacturers of containers by hand lamination.”
2. Page 174, lines 12-13. The following text in parentheses, “(workers from plants employing 50% to 100% laminators),” is deleted from the sentence: “Kolstad *et al.* (1995) reported significant risks of pancreatic cancer among individuals with probable high styrene exposures (workers from plants employing 50% to 100% laminators), and among individuals exposed to styrene for greater than one year.”

The following sentence is added: “The authors classified employees at companies with 50% or more of workers involved in reinforced plastics as probable high exposure, and most of the companies were boat yards or manufacturers of containers by hand lamination.”

3. Page 384, lines 7-9. The reference is added: “An alternative mechanism (Cruzan *et al.* 2002) 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.”

The following clarifications are made to the Final Report on Carcinogens Background Document for Styrene. New text is shown in italics.

1. The terms “statistically significant” and/or “statistically non-significant” and/or the *P* value are added to clarify the reported findings as follows:
 - Page xii, lines 19-22 and page 192, lines 14-17: “In the styrene monomer and polymer industries, the risk of lymphohematopoietic malignancies was also increased (*both statistically significant and statistically non-significant*) 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.”
 - Page xii, line 30 to page xiii, line 2 and page 192, lines 25 to 27: “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 (*P = 0.068*) in the multi-plant cohort.”
 - Page 178, lines 27-30: “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 *statistically non-significant* increased risk was also seen for myeloid leukemia with chromosomal aberrations in a nested case-control study of the Danish workers *based on small number of cases* (Kolstad *et al.* 1996).”

- Page 181, lines 19-24: “The nested case-control study from the Matanoski cohort of 58 lymphohematopoietic cases and 1,242 controls found two- to three-fold *statistically significant* 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 ($P = 0.023$) to styrene using the step-down regression analysis and taking into account butadiene exposure and other variables.
 - Page 184, lines 20-22: “A *statistically significant* 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).”
 - Page 184, lines 25-29: “*Statistically significant* 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 counts with varying environmental toxic releases].”
2. Page 360, lines 17-18. The following sentence is deleted: “However, most of the studies published prior to 1994 were negative while most of the studies published after 1994 were positive.”
 3. Table 5-18, page 367. The designation for Mutations – *In vivo* Humans is changed from “(+)” to “inconclusive.”