Styrene

CAS No. 100-42-5

Reasonably anticipated to be a human carcinogen

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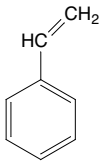

Carcinogenicity

Styrene is *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity from studies in experimental animals, and supporting data on mechanisms of carcinogenesis.

Cancer Studies in Humans

The limited evidence for the carcinogenicity of styrene in humans is based on studies of workers exposed to styrene that showed (1) increased mortality from or incidence of cancer of the lymphohematopoietic system and (2) increased levels of DNA adducts and genetic damage in lymphocytes from exposed workers. Elevated risks of lymphohematopoietic cancer were found among workers with higher exposure to styrene after an appropriate elapsed time since first exposure. In some studies, the risks increased with increasing measures of exposure, such as average exposure, cumulative exposure, or number of years since first exposure. However, the types of lymphohematopoietic cancer observed in excess varied across different cohort studies, and excess risks were not found in all cohorts. There is also some evidence for increased risks of esophageal and pancreatic cancer among styrene-exposed workers. Causality is not established, as the possibility that the results were due to chance or to confounding by exposure to other carcinogenic chemicals cannot be completely ruled out. However, a causal relationship between styrene exposure and cancer in humans is credible and is supported by the finding of DNA adducts and chromosomal aberrations in lymphocytes from styrene-exposed workers.

Most of the evidence in humans comes from occupational cohort studies in two major industries: (1) the reinforced-plastics industry and (2) the styrene-butadiene rubber industry. Studies of workers in a third industry, the styrene monomer and polymer industry, were not considered to be as informative, because they were limited by small numbers of cancer cases among exposed workers, and there was potential confounding by coexposure to benzene. Workers in the reinforced-plastics industry were exposed to the highest levels of styrene, and they had few other potentially carcinogenic exposures. However, the majority of the workers had short periods of employment. In the styrene-butadiene rubber industry, workers were exposed to lower levels of styrene than in the reinforced-plastics industry, but a large number of workers studied had long-enough follow-up times to permit detailed analysis of the incidences of lymphohematopoietic cancers. The main limitation of the studies in styrene-butadiene rubber workers is potential confounding by other exposures, principally to butadiene, which is a known human carcinogen associated with increased risk of leukemia (Grosse *et al.* 2007, NTP 2004a); exposures to butadiene and styrene are highly correlated in this industry.

The most informative studies in the reinforced-plastics industry were the two largest cohort studies: a Danish cohort of male workers (Kolstad *et al.* 1994, 1995) and a European multinational mortality cohort of predominantly male workers, which included a subset of the Danish workers (Kogevinas *et al.* 1994). In the styrene-butadiene industry, the major study was a large multi-plant cohort mortality study of male styrene-butadiene rubber workers in the United States and Canada (Graff *et al.* 2005, Delzell *et al.* 2006), which encompassed most of the workers from two earlier cohorts (a small study by Meinhardt *et al.* 1978 and a larger study by Matanoski *et al.* 1990). The studies in both industries included internal analyses (using unexposed members of the cohort as the comparison group); such analyses are less susceptible to confounding than those using external reference populations. Internal analyses were used to evaluate exposure-response relationships for cumulative exposure, average exposure, peak exposure (a measure of exposure intensity), or time since first exposure in the multinational cohort study of reinforced-plastics workers (Kogevinas *et al.* 1994) and in the multi-plant study of styrene-butadiene workers (Delzell *et al.* 2006). Without *a priori* knowledge, it is difficult to know which exposure metric is most appropriate for evaluating causality, so a positive relationship observed with any exposure metric is a concern. The studies also conducted standardized mortality ratio (SMR) or standardized incidence ratio (SIR) analyses, which compared observed with expected numbers of events (deaths or incident cases) based on national mortality or incidence rates. Two additional cohort studies of U.S. reinforced-plastics workers were less informative. A study by Ruder *et al.* (2004) had limited statistical power to detect positive associations between styrene exposure and uncommon types of cancer. A study by Wong *et al.* (1994) had a relatively large cohort and conducted internal analyses; however, the internal analyses were limited to exposure duration and cumulative exposure.

Lymphohematopoietic Cancer

Increased risks for leukemia, lymphoma, or all lymphohematopoietic cancer were found among styrene-exposed workers in both the reinforced-plastics and styrene-butadiene rubber industries. The evidence comes primarily from positive exposure-response relationships found in the multinational European study (reinforced-plastics workers) (Kogevinas *et al.* 1994) and the multi-plant cohort study of styrene-butadiene workers (Delzell *et al.* 2006) and is supported by findings of increased cancer risks among subgroups of workers with higher levels of styrene exposure or longer times since first exposure (Kogevinas *et al.* 1994, Kolstad *et al.* 1994). Although coexposure to butadiene is a concern in the styrene-butadiene industry, the finding of increased cancer risk in the reinforced-plastics industry, where such confounding is not an issue, suggests that styrene is a potential risk factor for lymphohematopoietic cancer. The types of lymphohematopoietic cancer observed in excess varied across different cohorts; a similar pattern has been observed for other epoxide-forming substances, such as 1,3-butadiene and ethylene oxide (see the profiles for those substances). Moreover, it is difficult to compare the risks for specific types of lymphohematopoietic cancer across studies, because (1) these cancers may have been grouped differently between studies or in the same study between different types of analyses (e.g., external and internal analyses in the study by Wong *et al.* 1994), (2) diagnoses based on death certificates may be inaccurate, and (3) lymphohematopoietic cancer classification and groupings have changed over time. In general, these limitations make it more difficult to see consistent associations between styrene exposure and specific types of lymphohematopoietic cancer across studies.

Reinforced-Plastics Industry

In the multinational study of reinforced-plastics workers, workers in the two highest categories of average styrene exposure had

significantly higher risks (or elevated risks approaching statistical significance) than did workers in the lowest exposure group for all lymphohematopoietic cancer (relative risk [RR] = 3.08, 95% confidence interval [CI] = 1.04 to 9.08, 13 cases with exposure of 120 to 199 ppm; RR = 3.59, 95% CI = 0.98 to 13.14, 8 cases with exposure ≥ 200 ppm). In addition, the risk of malignant lymphoma was significantly elevated in the second-highest exposure group (RR = 7.15, 95% CI = 1.21 to 42.11, 8 exposed cases). A fourfold higher risk of malignant lymphoma was also found for the highest-exposure group, but it was based on small numbers of exposed cases and was not statistically significant. Risks increased with increasing average exposure for all lymphohematopoietic cancer (P_{trend} = 0.019) and for malignant lymphoma (P_{trend} = 0.052). Time since first hire also was associated with lymphohematopoietic cancer (P_{trend} = 0.012) and malignant lymphoma (P_{trend} = 0.072); risk estimates for workers with the longest time since first hire compared with workers with the shortest time since first hire were 3.97 (95% CI = 1.30 to 12.13, 9 exposed cases) for all lymphohematopoietic cancer and 5.16 (95% CI = 0.90 to 29.47, 4 exposed cases) for malignant lymphoma (Kogevinas *et al.* 1994). No significant relationship with cumulative exposure was observed, although statistically nonsignificant elevated risks for lymphoma were found for all groups with cumulative exposure greater than 75 ppm. The proportion of short-term workers was higher among the workers with the highest exposure levels (laminators); therefore, measures of exposure intensity (such as average exposure level) may be more informative than measures of exposure duration for evaluating risks.

Among Danish reinforced-plastics workers, the incidence of leukemia was significantly elevated for workers with earlier dates of first exposure (1964 to 1970, during which time the highest exposure levels occurred) (Kolstad *et al.* 1994). Significantly elevated risks were also found among workers with at least ten years since first employment; within this group, the increased risks were concentrated among short-term workers (those workers with exposure duration of less than one year). The findings for leukemia were similar in the internal analyses using unexposed workers as controls for short-term workers, thus helping to rule out confounding by socioeconomic status or lifestyle factors of the short-term workers.

Neither of the two U.S. cohort studies of reinforced-plastics workers found a significant association between styrene exposure and lymphohematopoietic cancer; however, neither study evaluated risk by average exposure intensity, and the smaller study (Ruder *et al.* 2004) had very limited statistical power to detect an association. In the larger U.S. study (Wong *et al.* 1994), no association was found between cumulative exposure or duration of exposure and all lymphohematopoietic cancer, non-Hodgkin's lymphoma, or leukemia. The analysis included both exposure measures, which are highly correlated with each other; this may have reduced the statistical power to detect an association (IARC 2002).

Styrene-Butadiene Rubber Industry

The multi-plant cohort study of male styrene-butadiene rubber workers found significantly increased risks (SMRs) of non-Hodgkin's lymphoma (NHL), NHL-chronic lymphocytic leukemia (NHL-CLL), and leukemia (overall and specific types) among subgroups of workers with long duration of employment (> 10 years) and long time since first exposure (20 to 29 years or ≥ 30 years), in specific job categories, and with the highest levels of cumulative exposure to styrene (Graff *et al.* 2005, Sathiakumar *et al.* 2005, Delzell *et al.* 2006).

In an attempt to disentangle the effects of styrene from those of butadiene, internal analyses were conducted for quartiles of cumulative exposure or exposure to periodic spikes of high styrene concentrations (styrene peaks, defined as ≥ 50 ppm) involving statistical models with (1) styrene exposure only, (2) styrene and butadiene exposure, and (3) styrene and butadiene exposure plus dermal exposure to dimethyldithiocarbamate (DMDTC). (The relevance of including DMDTC in these models is not clear, because there is no independent evidence that DMDTC is carcinogenic in animals or humans.) The number of cases at each exposure level was small, which limited the power to detect statistically significant risk estimates. No trend analyses were reported. The analyses suggested an exposure-response relationship between NHL and NHL-CLL combined and exposure to styrene that was not explained by exposure to butadiene. The relative risk of NHL or NHL-CLL increased with increasing level of cumulative exposure to styrene and was not attenuated by control for butadiene exposure. However, the relative risk reached statistical significance only for the highest styrene exposure level in the styrene-only model and only for NHL-CLL combined. Exposure to butadiene was not associated with risk of NHL or NHL-CLL (Graff *et al.* 2005, Delzell *et al.* 2006).

Evidence for an association between styrene exposure and leukemia comes from analyses of cancer among workers exposed to styrene peaks. The relative risk of leukemia increased with exposure to increasing numbers of styrene peaks in all three chemical models and was significantly elevated at the two highest styrene exposure levels with control for butadiene exposure. The relative risk of leukemia also increased with increasing cumulative styrene exposure, but the response was attenuated by control for butadiene exposure, and no association remained after additional control for DMDTC.

A nested case-control study from the Matanoski cohort also found significantly increased risks of all lymphohematopoietic cancer (P = 0.001) and of lymphoma (P = 0.020) (International Classification of Disease codes 200 and 202, which are the same codes as for NHL) with exposure to styrene (1-ppm time-weighted average, compared with 0 ppm) in a statistical model that accounted for exposure to butadiene. Although the study population overlapped with that of the multi-plant cohort, it provided supporting evidence for the increased risk of lymphoma reported by Delzell *et al.,* because it used a different exposure assessment (based on measurements) and a different statistical model (Matanoski *et al.* 1997).

Cancer at Other Tissue Sites

Studies in the reinforced-plastics industry provided evidence that suggests a possible association between styrene exposure and cancer of the esophagus or pancreas. Mortality from esophageal cancer was increased in two of the four studies (Ruder *et al.* 2004, Wong *et al.* 1994), and a third study found a statistically nonsignificant increased risk among the workers with higher cumulative exposure (Kogevinas *et al.* 1994). For pancreatic cancer, increased risks were suggested in the cohort studies. Internal analyses of the Danish cohort found a significant risk of pancreatic cancer (incidence) among workers classified as having "probable high exposure" (Kolstad *et al.* 1995). Statistically nonsignificant increased risks of pancreatic cancer mortality were reported by the two U.S. cohort studies (Ruder *et al.* 2004, Wong *et al.* 1994) and for workers with higher cumulative exposure in the European study (Kogevinas *et al.* 1994). There was some evidence of an exposure-response relationship for pancreatic cancer; cancer risk increased with increasing cumulative exposure in the European multi-plant cohort (P_{trend} = 0.068) (Kogevinas *et al.* 1993, 1994). No excess mortality from esophageal or pancreatic cancer was found in studies of styrene-butadiene rubber workers; however, the only analysis reported was the SMR for the entire multi-plant cohort (Delzell *et al.* 2006).

Genetic Damage

DNA adducts (primarily, N^2-guanine and O^6-guanine, but also βN1-adenine adducts) were found in circulating white blood cells in many studies of styrene-exposed workers employed mainly in the reinforced-plastics industry; levels of O^6-guanine were five- to seven-fold higher among styrene-exposed workers than controls (Vodicka *et al.* 2006a, Boffetta *et al*. 2009). In most studies in workers, single-strand DNA breaks showed exposure-related increases (Brenner *et al.* 1991, Maki-Paakkanen *et al.* 1991, Vodicka *et al.* 2006a). A meta-analysis of 22 studies found a positive association (weighted frequency ratio = 2.18, 95% CI = 1.52 to 3.13) between styrene exposure level and chromosomal aberration frequency when exposure levels were dichotomized as greater than or less than a threshold value of 30 ppm for an 8-hour time-weighted average (Bonassi *et al.* 1996).

Cancer Studies in Experimental Animals

Styrene caused lung tumors in several strains of mice and by two different routes of exposure. The most robust studies are two-year studies of inhalation exposure in CD-1 mice (Cruzan *et al.* 2001) and oral exposure (by stomach tube) in $B6C3F_1$ mice (NCI 1979). Inhalation exposure caused benign lung tumors (alveolar/bronchiolar adenoma) and increased the combined incidence of benign and malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma) in CD-1 mice of both sexes; in females, it also increased the separate incidence of malignant lung tumors. In male $B6C3F_1$ mice, oral exposure to styrene increased the combined incidence of benign and malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma), and a positive dose-response trend was observed (NCI 1979).

These findings are supported by findings of lung tumors in both sexes of O20 mice exposed to styrene (Ponomarkov and Tomatis 1978). In O20 mice, a single dose of styrene was administered to pregnant dams on gestational day 17, and pups were exposed orally once a week for 16 weeks after weaning. A significantly increased incidence and earlier onset of benign and malignant lung tumors combined (adenoma and carcinoma) occurred in mice of both sexes as early as 16 weeks after weaning. In a similar study with C57Bl mice administered a much lower dose of styrene, lung-tumor incidence was not significantly increased. In short-term studies, oral exposure to styrene caused cytotoxicity and increased cell replication in the mouse lung, supporting the findings of lung tumors following oral exposure to styrene in longer-term studies (Green *et al.* 2001).

The evidence from studies in rats is insufficient for reaching a conclusion concerning the carcinogenicity of styrene. Lung tumors were not observed in rats (IARC 2002); however, findings for mammary-gland tumors were equivocal. The incidence of mammary-gland tumors was increased in female Sprague-Dawley rats exposed to styrene in the drinking water (mammary fibroadenoma; Huff 1984) or by inhalation (malignant tumors; Conti *et al.* 1988), but decreased incidences of mammary-gland tumors (adenocarcinoma) were reported in another inhalation-exposure study of rats of the same strain (Cruzan *et al.* 1998).

Metabolism of Styrene

Styrene can be absorbed and widely distributed throughout the body through inhalation, ingestion, or skin contact, but the most important route of occupational exposure is inhalation (IARC 2002). Styrene is metabolized primarily (over 90%) to the genotoxic metabolite styrene-7,8-oxide, which can be detoxified by glutathione conjugation or conversion to styrene glycol by microsomal epoxide hydrolase. Pharmacokinetic models predict the concentration of styrene in the lung (Filser *et al.* 2002) or terminal bronchioles (Sarangapani *et al.* 2002) to be higher in mice than in rats and higher in rats than in humans. Systemic distribution of styrene-7,8-oxide in workers has been demonstrated from measurements of styrene-7,8-oxide-based hemoglobin adducts in erythrocytes and DNA adducts in lymphocytes (Tornero-Velez *et al.* 2001, Vodicka *et al*. 2003, 2006a). Further oxidation of styrene glycol produces mandelic acid and phenylglyoxylic acid, the major metabolites identified in the urine of styrene-exposed workers (Manini *et al.* 2002). Because styrene-7,8-oxide contains a chiral carbon, it and some subsequent styrene metabolites can exist as either R or S enantiomers. A second, minor pathway of styrene metabolism involves oxidation of the aromatic ring resulting in formation of 4-vinylphenol, presumably via the arene intermediate styrene-3,4-oxide, which has been detected in humans (Pfäffli *et al.* 1981, Manini *et al.* 2003) and rats (Bakke and Scheline 1970) and whose occurrence in mice *in vivo* was implicated by indirect measures (Boogaard *et al.* 2000).

Styrene is metabolized primarily in the liver and the lung. In mice, the Clara cell is regarded as the major lung-cell type in which styrene is activated to styrene-7,8-oxide following inhalation exposure (Hynes *et al.* 1999). The initial step in styrene metabolism is catalyzed by cytochromes P450, and there are tissue-specific differences in the enzymes responsible for styrene oxidation. In mice, Cyp2e1 predominates in the liver, and Cyp2f2 in the lung (Carlson 1997, 2004, Vodicka *et al*. 2006a). In humans, CYP2A13, CYP2F1, CYP1A2, CYP2C8, CYP2A6, and CYP2E1 are active in metabolizing styrene to styrene glycol in the lung, and CYP2B6 and CYP2E1 are most active in the liver (Nakajima *et al.* 1994, IARC 2002, Fukami *et al.* 2008). Human CYP2F1 (equivalent to Cyp2f2 in mice and CYP2F4 in rats) has been shown to metabolize styrene in vitro (Nakajima *et al.* 1994). In general, expression of CYP enzymes is more widely distributed in the human lung than in the lungs of experimental animals, where expression is concentrated in Clara cells, type II alveolar cells, and alveolar macrophages. CYP2B6 is expressed in human Clara cells, and CYP2E1 in human bronchial, bronchiolar, and alveolar epithelium, alveolar macrophages, and lung tumors (Kivistö *et al.* 1995, Hukkanen *et al.* 2002). CYP2E1 is also expressed in lymphocytes (Siest *et al.* 2008), and CYP2E1 protein and activity were detected in human hematopoietic stem cells (Kousalova *et al.* 2004).

Because many of the enzymes involved in styrene metabolism are polymorphic, individuals may differ in their susceptibility to styrene-induced toxicity. Some studies have found that polymorphisms in glutathione S-transferase mu 1 influence excretion of styrene metabolites (De Palma *et al.* 2001, Haufroid *et al.* 2002, Teixeira *et al.* 2004); however, studies evaluating genotoxicity and polymorphisms in genes involved in either styrene metabolism or DNA repair have not clearly identified specific polymorphisms related to genotoxic effects (Godderis *et al.* 2006, Migliore *et al.* 2006, reviewed by Vodicka *et al.* 2006a).

Studies on Mechanisms of Carcinogenesis

The mechanisms of styrene carcinogenicity are not fully understood. The primary metabolite of styrene, styrene-7,8-oxide, is listed in the Report on Carcinogens as *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals. Oral exposure to styrene-7,8-oxide caused forestomach tumors in rats and mice and liver tumors in male mice (see the profile for styrene-7,8-oxide, NTP 2004b).

The proposed mechanisms for the carcinogenicity of styrene include both genotoxic and non-genotoxic pathways, which are not necessarily mutually exclusive. Most of the mechanistic studies have focused on either general genotoxicity or issues considered relevant to the mouse lung tumors, and there has been little research on mechanisms specific to lymphohematopoietic cancer in humans. Possible

modes of action for styrene-induced carcinogenicity involve (1) genotoxicity (relevant to all types of cancer), (2) cytotoxic effects of styrene metabolites in the mouse lung, and (3) immunosuppression (relevant to lymphohematopoietic cancer).

Genotoxicity

Most of the genetic damage associated with styrene exposure is thought to be due to styrene-7,8-oxide. The predominant DNA adducts formed as a result of styrene-7,8-oxide exposure occur at the N7, N^2, and O^6 positions of guanine (these have been detected in cells); however, styrene-7,8-oxide adducts can also form at the N1, N3, and N^6 positions of adenine, the N3, N^4, and O^2 positions of cytosine, and the N3 position of thymine. N7-adducts are formed in the largest amounts but are the least persistent (i.e., they are either repaired or lost), whereas O^6-adducts are formed in the smallest amounts but are the most persistent. Other than the N7-guanine and N3-adenine adducts, the styrene-7,8-oxide–DNA adducts listed above are considered promutagenic, because they can interfere with base pairing and lead to miscoding during DNA replication. The major styrene-7,8-oxide adduct at N7-guanine may also be promutagenic, because it can undergo spontaneous or glycosylase-mediated depurination, thus creating abasic sites that promote coding errors during DNA replication (Vodicka *et al.* 2006a). Styrene-7,8-oxide, without metabolic activation, is mutagenic in most *in vitro* systems, causing a variety of transition and transversion mutations (Bastlová and Podlutsky 1996). Both styrene and styrene-7,8-oxide caused cytogenetic effects (sister chromatid exchange, chromosomal aberrations, and micronucleus formation) in human lymphocytes or other mammalian cells *in vitro.* In mice and rats exposed to styrene *in vivo,* N7-guanine, O^6-guanine, and N1-adenine adducts were detected in liver and lung cells (Pauwels *et al.* 1996, Boogaard *et al.* 2000b, Vodicka *et al.* 2001, 2006a,b). Most studies in mice also found single-strand DNA breaks following exposure to styrene-7,8-oxide or styrene (Walles and Orsen 1983, Vaghef and Hellman 1998, Vodicka *et al*. 2001), and the cytogenetic effect reported most consistently was sister chromatid exchange (Conner *et al.* 1979, 1980, Sharief *et al.* 1986, Kligerman *et al.* 1992, 1993, Simula and Priestly 1992; reviewed by IARC 1994, 2002 and Scott and Preston 1994).

Styrene-7,8-oxide was measured in the blood of styrene-exposed workers, and several different styrene-7,8-oxide–based DNA adducts were detected in their lymphocytes. Styrene-7,8-oxide–DNA adducts identified in exposed workers include O^6-guanine, N1-adenine, and N^2-guanine. Styrene-7,8-oxide adducts were also detected in human volunteers exposed to styrene under conditions designed to eliminate or minimize non-enzymatic oxidation to styrene-7,8-oxide (Johanson *et al.* 2000). Adduct studies in workers showed that a DNA-reactive intermediate of styrene metabolism circulates in the blood of styrene-exposed humans (Vodicka *et al.* 2006a). The most consistent cytogenetic effects in styrene-exposed workers were single-strand DNA breaks and chromosomal aberrations (Anwar and Shamy 1995, Bonassi *et al.* 1996, Lazutka *et al.* 1999, Somorovská *et al.* 1999, reviewed by Cohen *et al.* 2002).

Lung Cytotoxicity in Mice

Cytotoxicity can cause regenerative hyperplasia, leading to the promotion of spontaneous or styrene-induced mutations and tumor formation. Styrene caused lung tumors and pulmonary toxicity in mice but did not cause lung tumors in rats (Cruzan *et al*. 1998, 2001). The induction of lung tumors in mice but not in rats has also been observed in studies of exposure to epoxides and other epoxide-forming chemicals, including the known human carcinogens vinyl chloride, 1,3-butadiene, and ethylene oxide (NTP 2004a,b; see the profiles for those substances).

Although several studies found no evidence of toxicity in the lungs of rats exposed to styrene (Cruzan *et al.* 1997, 1998, Green *et al*. 2001, Gamer *et al.* 2004), one study reported toxic effects on bronchiolar and alveolar type II cells in Sprague-Dawley rats exposed to styrene by inhalation or intraperitoneal injection (Coccini *et al.* 1997). Alveolar/bronchiolar hyperplasia from styrene exposure has been hypothesized to play a role in the development of lung tumors in mice. Effects of repeated styrene exposure in mice included focal crowding of bronchiolar cells, bronchiolar epithelial hyperplasia, and bronchiolar/alveolar hyperplasia (Cruzan *et al.* 2001). Interspecies differences in lung toxicity are proposed to result from differences in the extent of metabolism of styrene to ring-oxidized metabolites by Cyp2f in the Clara cells (Cruzan *et al.* 2002, 2009).

Indirect data supporting the role of Cyp2f in styrene-induced lung toxicity comes from short-term intraperitoneal-injection studies with wild-type and Cyp2e1 knock-out mice, which showed similar lung toxicity (Carlson 2004). Also, the cytotoxic effects of styrene and tumor formation were seen primarily in respiratory tissues that are high in Cyp2f isoforms, and Cyp2f inhibitors prevented cytotoxicity (Cruzan *et al.* 2002). Styrene-7,8-oxide, 4-vinylphenol, and 4-vinylphenol metabolites can be formed by Cyp2f2. Metabolites formed from ring oxidation, including 4-vinylphenol, occur at several-fold higher levels in mice than in rats (Boogaard *et al.* 2000a, Cruzan *et al.* 2002). Some data suggest that 4-vinylphenol is more toxic than styrene-7,8-oxide in mouse lung; however, the two metabolites were tested in separate experiments in two different mouse strains (Gadberry *et al.* 1996, Carlson 2002). Short-term toxicity studies of 4-vinylphenol in wild-type and Cyp2e1 knock-out mice and studies with CYP inhibitors suggest that metabolites of 4-vinylphenol are responsible for its lung and liver toxicity in mice (Carlson 2002, Vogie *et al*. 2004).

Immunosuppression

The mechanism for styrene-induced lymphohematopoietic cancer is not known. As discussed above, CYP2E1 is expressed in lymphocytes (Siest *et al.* 2008), and CYP2E1 protein and activity were detected in human hematopoietic stem cells (Kousalova *et al.* 2004), suggesting that styrene can be metabolized to styrene-7,8-oxide in the target tissues. Moreover, studies on genotoxicity and oxidative stress in styrene-exposed workers indicated that styrene causes DNA and chromosomal damage in peripheral blood lymphocytes. Immunosuppression has been proposed as a mechanism for solvent-induced lymphoma (Vineis *et al.* 2007). Styrene-exposed workers had decreased numbers of activated helper T-cell lymphocytes, suggesting that styrene exposure can cause immunosuppression; however, this study was limited in size, and the workers were exposed to other agents (Biró *et al.* 2002). In a review of studies in experimental animals and humans, Veraldi *et al.* (2006) concluded that there was "immediate" evidence for the immunotoxicity of styrene oxide, and that the main immunotoxic effect was immunosuppression.

Summary

Although styrene disposition differs quantitatively among species, no qualitative differences between humans and experimental animals have been demonstrated that contradict the relevance of cancer studies in rodents for evaluation of human hazard. Detection of styrene-7,8-oxide–DNA adducts at base-pairing sites and chromosomal aberrations in lymphocytes of styrene-exposed workers supports the potential human cancer hazard from styrene through a genotoxic mode of action.

Properties

Styrene is an aromatic hydrocarbon that occurs as a colorless or yellowish viscous liquid with a sweet, floral odor (HSDB 2008). It has a flash point of 34°C (closed cup), a lower explosive limit of 0.9% to 1.1% v/v, an 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 as a result of the formation of peroxides. Styrene may polymerize when contaminated by oxidizing agents or halides, or when heated, and it emits acrid fumes upon decomposition (SPA 2008, Akron 2010). Styrene usually is stabilized for safe storage, transport, and use by an inhibitor, commonly *p-tert*-butylcatechol (HSDB 2008). Other typical impurities are ethylbenzene, polymer content, aldehydes, peroxides (as H_2O_2), benzene, sulfur, and chlorides. Physical and chemical properties of styrene are listed in the following table.

Property	Information
Molecular weight	104.2
Specific gravity	0.906 at 20°C
Melting point	–31°C
Boiling point	145°C
Log K_{ow}	2.95
Water solubility	310 mg/L at 25°C
Vapor pressure	6.4 mm Hg at 25°C
Vapor density relative to air	3.6

Source: HSDB 2008.

Use

Styrene is an important industrial chemical, used in the synthesis and manufacture of polystyrene and hundreds of different copolymers, as well as numerous other industrial resins (Guest 1997). Styrene producers sell styrene monomer to companies that use styrene to make various compounds and resins. Fabricators then process the resins into a wide variety of products (Cohen *et al.* 2002). Roughly 99% of the industrial resins produced from styrene can be grouped into six major categories: polystyrene (50%), styrene-butadiene rubber (15%), unsaturated polyester resins (glass reinforced) (12%), styrene-butadiene latexes (11%), acrylonitrile-butadiene-styrene (10%), and styrene-acrylonitrile (1%). Another minor category of use is unsaturated polyester resins (not reinforced) (Luderer *et al.* 2005).

Polystyrene is used extensively in the manufacture of plastic packaging, thermal insulation in building construction and refrigeration equipment, and disposable cups and containers. Styrene polymers and copolymers are also increasingly used to produce various housewares, food containers, toys, electrical devices, automobile body parts, corrosion-resistant tanks and pipes, various construction items, carpet backings, house paints, computer printer cartridges, insulation products, wood-floor waxes and polishes, adhesives, putties, personal-care products, and other items, and they are used in paper processing (IARC 2002, Luderer *et al.* 2005, NLM 2008).

Styrene-butadiene rubber is the most widely used synthetic rubber in the world (ICIS 2008). Over 70% of styrene-butadiene rubber is consumed in the manufacture of tires and tire products; however, non-tire uses are growing, with applications including conveyor belts, gaskets, hoses, floor tiles, footwear, and adhesives.

Another major use of styrene is as a cross-linking agent in polyester resins used in gel-coating and laminating operations in the production of glass-fiber-reinforced plastic products such as boats, bathtubs, shower stalls, tanks, and drums (Miller *et al.* 1994, EPA 1997). The resins generally contain between 30% and 50% styrene by weight (EPA 1997).

Production

There are two commercially viable methods of producing styrene (ATSDR 1992, HSDB 2008). The most common process, which accounts for over 90% of total world styrene production, involves catalytic dehydrogenation of ethylbenzene. The second process involves oxidation of ethylbenzene to its peroxide, which is then reacted with propylene to produce propylene oxide and α-methylphenyl carbinol. The carbinol is then dehydrated to produce styrene. U.S. production of styrene has risen fairly steadily since 1960. Between 1960 and 2006, estimated production ranged from a low of 1,740 million pounds in 1960 to a high of 11,897 million pounds in 2000. In 2006, eight U.S. manufacturers produced an estimated 11,387 million pounds of styrene; the three largest producers accounted for 54% of production. U.S. consumption of styrene in 2006 was 9,600 million pounds, over 99% of which was consumed in the production of polymers and copolymers (Berthiaume and Ring 2006). U.S. imports and exports of styrene increased steadily from 1975 through 2007, from 7 million pounds to 1,475 million pounds for imports and from 574 million pounds to 4,200 million pounds for exports (Berthiaume and Ring 2006, USITC 2008a,b).

Exposure

Exposure to styrene can occur in both occupational and nonoccupational settings. However, workers in certain occupations potentially are exposed to much higher levels of styrene than the general population. The greatest source of exposure for the general population is cigarette smoking, and daily styrene intake by the nonsmoking population is expected to be orders of magnitude lower than daily intakes for workers in occupations with high styrene exposure levels (Cohen *et al.* 2002, IARC 2002).

Exposure of the General Population

Styrene exposure to the general population can occur through environmental contamination. For the non-smoking general population, inhalation of indoor air and ingestion of food resulted in the highest daily styrene intakes (IARC 2002). Styrene has been measured in outdoor air, but higher levels generally are found in indoor air, drinking water, groundwater, surface water, soil, and food. Styrene can be emitted to the air from industrial production and use of styrene and styrene-based polymers and copolymers, motor-vehicle emissions and other combustion processes, offgassing of building materials and consumer products, and cigarette smoking (ATSDR 2010, IARC 1994). Numerous spills containing styrene have been reported to the National Response Center since 1990, and these spills have the potential to contaminate air, water, soil, and food supplies (NRC 2008). Uptake of styrene by biological organisms is expected to be low; however, styrene has been detected in fish and other aquatic organisms (Howard 1989, ECB 2002, HSDB 2008).

Food can contribute to styrene exposure (Lickly *et al.* 1995a, Tang *et al.* 2000, Cohen *et al.* 2002, Holmes *et al.* 2005). Styrene has been detected in a wide range of foods and beverages, with the highest measured levels occurring in unprocessed, raw cinnamon, possibly resulting from the natural degradation of cinnamic acid derivatives (IARC 1994). Styrene also occurs at very low concentrations in many agricultural food products; however, it is not known whether the styrene is produced endogenously or results from environmental contamination (Tang *et al.* 2000). The presence of styrene in packaged foods is due primarily to leaching of monomer from polystyrene containers (Howard 1989, ATSDR 2010). The rate of migration of styrene monomer from polystyrene containers is determined mainly by the lipophilicity of the food, surface area of the container per volume of

food, duration of contact, and food temperature (ATSDR 2010, Lickly *et al.* 1995b, ECB 2002, Choi *et al.* 2005).

In a study comparing styrene intake from various sources, estimated daily intake for adults was lowest from polluted drinking water and highest from cigarette smoke, polluted urban air, and indoor air (Fishbein 1992). Estimated daily styrene intake for the Canadian general population from sources other than smoking was less than 0.8 μg/kg of body weight for children and less than 0.4 μg/kg for adults, but estimated daily intake for cigarette smokers was as high as 3.5 μg/kg (CEPA 1993). While this study demonstrated that inhalation of both indoor and outdoor air and ingestion of food are important sources of exposure for nonsmokers, it also estimated that exposure from smoking cigarettes was roughly 10 times that from all other routes (indoor and outdoor air, drinking water, soil, and food) combined. Other studies estimated that styrene exposure of smokers was six times that of nonsmokers (Cohen *et al.* 2002) and that up to 15% of nonsmokers' styrene exposure could be attributed to environmental tobacco smoke (Miller *et al.* 1998).

In a 1982 study by the U.S. Environmental Protection Agency, styrene was detected in all of eight human-breast milk samples from women in four U.S. cities and in all of an unspecified number of wet adipose tissue samples (Howard 1989). Styrene also was detected in the general population at mean concentrations of 0.4 μg/L in blood and 0.7 to 1.6 μg/m^3 in exhaled breath (ATSDR 2010). Blood styrene levels were assessed in the Priority Toxicant Reference Range Study conducted as part of the Centers for Disease Control and Prevention's Third National Health and Nutrition Examination Survey. Of 624 samples, 78 (12.5%) contained no detectable styrene, and 546 contained styrene at concentrations ranging from 0.019 to 4.006 μg/L; the mean concentration for all 624 samples was 0.07 μg/L, the median was 0.04 μg/L, and the 95th percentile value was 0.18 μg/L (Ashley *et al.* 1994, Sexton *et al.* 2005).

Occupational Exposure

Workers can be exposed to styrene during production of styrene monomer, polystyrene and various styrene copolymers, glass-fiber-reinforced plastics, and styrene-butadiene rubber; exposure can also occur in other miscellaneous occupations (ATSDR 2010, IARC 2002).

The highest levels of occupational exposure to styrene occur in the fabrication of products such as boats, car and truck parts, tanks, bathtubs, and shower stalls from glass-fiber-reinforced polyester composite plastics (IARC 2002). Historically, the highest styrene exposure levels for reinforced-plastics workers were in the range of several hundred parts per million; however, estimated exposure levels have decreased by a factor of 10 over the past several decades as a result of improved work practices and products (Kolstad *et al.* 2005). In general, the average exposure levels reported since the 1980s have been less than 100 ppm. In 2006, the U.S. Bureau of Labor Statistics estimated that 32,510 workers were employed as Fiberglass Laminators and Fabricators (defined as "laminate layers of fiberglass on molds to form boat decks and hulls, bodies for golf carts, automobiles, or other products"). Ship and Boat Building was the largest subcategory in this Standard Occupational Classification segment, with 12,910 employees (BLS 2007). Workers in the reinforced-plastics industry are potentially exposed to styrene-7,8-oxide, as well as styrene, but at levels 2 to 3 orders of magnitude lower than styrene (Serdar *et al.* 2006).

Styrene exposure levels are generally lower in the styrene-butadiene rubber and the styrene monomer and polymer industries than in the reinforced-plastics industry; however, significant exposure of workers still can occur. Reported mean exposure levels for these industries generally have been less than 20 ppm. No data were found on the numbers of employees in these industries. As in the reinforced-plastics industry, styrene exposure levels in these industries have declined over the past several decades (Macaluso *et al.* 1996, IARC 2002).

Low levels of styrene (usually in the low parts-per-billion range) have been reported in a variety of other occupational settings, including nuclear power plants, photocopy centers, a petrochemical complex, printing plants, wood surface-coating operations, tollbooths, and a waste incinerator, and during the production of PVC film (Kim *et al.* 2003, Bakoğlu *et al.* 2004, Leung *et al.* 2005, Sapkota *et al.* 2005, Thorud *et al.* 2005, Chan *et al.* 2006, Hsieh *et al.* 2006, Lee *et al.* 2006). Levels in the low parts-per-million range were measured in a sculpture class where polyester resins were used, during the production of buttons, and during firefighting. Higher levels were seen during the production or use of paints and putties (exceeding 20 ppm), for taxidermists (up to 70 ppm), and during the manufacture of cooking ware (up to 186 ppm) (IARC 2002).

Regulations

Coast Guard, Department of Homeland Security

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

Department of Transportation (DOT)

Styrene is considered a hazardous material, and special requirements have been set for marking, labeling, and transporting this material.

Environmental Protection Agency (EPA)

Clean Air Act

Mobile Source Air Toxics: Listed as a mobile source air toxic for which regulations are to be developed.
National Emissions Standards for Hazardous Air Pollutants: Listed as a hazardous air pollutant.
New Source Performance Standards: Manufacture of styrene is subject to certain provisions for the control of volatile organic compound emissions.

Clean Water Act

Designated a hazardous substance.

Comprehensive Environmental Response, Compensation, and Liability Act

Reportable quantity (RQ) = 1,000 lb.

Emergency Planning and Community Right-To-Know Act

Toxics Release Inventory: Listed substance subject to reporting requirements.

Safe Drinking Water Act

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

Food and Drug Administration (FDA)

Maximum permissible level in bottled water = 0.1 mg/L.
The food additive poly(2-vinylpyridine-co-styrene) may be safely used as a nutrient protectant in feed for beef cattle and dairy cattle and replacement dairy heifers, with residual styrene levels not to exceed 200 ppb.
Polystyrene basic polymers used as components of articles intended for use in contact with food shall contain not more than 1% by weight of total residual styrene monomer (0.5% by weight for certain fatty foods).
Rubber-modified polystyrene basic polymers used as components of articles intended for use in contact with food shall contain not more than 0.5% by weight of total residual styrene monomer.
Styrene-maleic anhydride co-polymers may be used as articles or as components of articles intended for use in contact with food provided that conditions are met, including residual styrene monomer levels not exceeding 0.3% by weight.
Styrene-acrylic co-polymers may be used as components of the food-contact surface of paper and paperboard provided that certain conditions are met, including residual styrene monomer levels not exceeding 0.1% by weight.

Occupational Safety and Health Administration (OSHA)

While this section accurately identifies OSHA's legally enforceable PELs for this substance in 2010, specific PELs may not reflect the more current studies and may not adequately protect workers.
Acceptable peak exposure = 600 ppm (5-min maximum peak in any 3 h).
Ceiling concentration = 200 ppm.
Permissible exposure limit (PEL) = 100 ppm.

Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH)

Threshold limit value – time-weighted average (TLV-TWA) = 20 ppm.
Threshold limit value – short-term exposure limit (TLV-STEL) = 40 ppm.

Biological exposure indices: Mandelic acid plus phenylglyoxylic acid in urine, end of shift = 400 mg/g of creatinine; styrene in venous blood, end of shift = 0.2 mg/L.

National Institute for Occupational Safety and Health (NIOSH)

Immediately dangerous to life and health (IDLH) limit = 700 ppm.
Short-term exposure limit (STEL) = 100 ppm.
Recommended exposure limit (REL) = 50 ppm.

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