Styrene-7,8-oxide and Styrene

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Citation for most recent IARC review
IARC Monograph 82, 2002

Current evaluation:

Styrene is *possibly carcinogenic to humans (Group 2B)*. The Working Group found *limited evidence* in humans and *limited evidence* in experimental animals for the carcinogenicity. Evidence from mechanistic studies did not contribute in making the overall classification decision.

Styrene-7,8-oxide is *probably carcinogenic to humans* (*Group 2A*). The Working Group found inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity. In making the overall evaluation, the Working Group took into consideration supporting evidence that styrene-7 ,8-oxide: (i) forms covalent adducts with DNA in humans, rats and mice; (ii) induces gene mutation in bacteria and rodent cells in vitro; (iii) induces chromosomal aberrations, micronuclei and sister chromatid exchange in human cells in vitro; and (iv) induces chromosomal aberrations and sister chromatid exchange in mice in vivo.

Recent Authoritative Review

The National Toxicology Program recently finalized a review of styrene (NTP, 2008). The review covered use, exposure, and evidence for carcinogenicity from epidemiology, animal, mechanistic and other relevant studies. Except where citations are given, the discussion below relies on the NTP review. The NTP review was finalized after peer review by the "Report on Carcinogens Expert Panel for Styrene" The Panel made conclusions about the evidence for the carcinogenicity of styrene. The Panel found limited evidence of carcinogenicity in humans and sufficient evidence in animals. Major considerations in the Panel's recommendation included the established animal carcinogenicity and genotoxicity of the metabolite styrene-7,8-oxide, and the evidence for styrene-related DNA adducts and cytogenetic effects in styrene-exposed workers. In writing this summary we relied on existing authoritative reviews such as the NTP document, which has been subjected to careful peer review, exhaustive fact

checking and public comment. Except where citations are given, the discussion relies on the NTP review.

Exposure and biomonitoring:

Exposure to styrene is common in the general population and in occupational settings. Since the release of the Monograph, a number of publications report on environmental levels of styrene.

There are a number of emission sources of styrene, which on average occurs in the United States in sub-ppb levels in outdoor and indoor air, with indoor concentrations generally higher than outdoor. Styrene occurs in tobacco and marijuana smoke (Moir et al., 2007); the smoker is exposed as well as those exposed to his or her smoke. Styrene is released in wood burning, fuel combustion, and a number of industrial sources. Styrene is used in the manufacture of polystyrene and numerous copolymers and resins, and emissions result from manufacture and fabrication. In the United States, styrene ranked fifth in fugitive air emissions in 2005. Besides smoke sources, indoor sources include consumer product off-gassing of residual styrene monomer in polymer and copolymer materials. This offgassing results in higher levels indoors than outdoors. Higher levels have recently been found inside stores in some metropolitan US cities. In personal monitoring studies, indoor sources produced greater exposures than outdoor. In a recent study in Leipzig, indoor sources dominated as well, with greater contributions to personal exposure from home than from office buildings (Gokhale et al., 2008).

Styrene exposures via tap water are currently low in the United States.

Styrene migrates into food packaging into food. There are few new references on food migration since the publication of the IARC Monograph. The United States Food and Drug Administration includes styrene as a chemical it monitors in its Total Diet Study, and there are recent data. Levels of styrene of 10 ppb and above are common in a variety of food products. There also can be non-residue sources of food exposure. One recent paper also demonstrated that certain food molds produced styrene in foods.

For the general population, aside from tobacco smoke, food appears to be the largest source of exposure, followed by indoor air. For smokers, the largest source is cigarette smoke. Styrene was detected by the United States Centers for Disease Control and Prevention (US CDC) in 87.5% of blood samples from a group 624 individuals aged 20-59 year, sampled in the late 1980's and early 1990's (http://ntp.niehs.nih.gov/files/Styrene_Background_Document_(9-29-08)F%5B1%5D.pdf, p.42). The highest value measured was 4 µg/L and the median was 0.04 µg/L. The caveat for this study was that: "It is important to note that because this study was conduced with a nonstatistical subsample of NHANES III participants, statistical weights cannot be assigned, and estimates for the total U.S. population therefore cannot be calculated (NCHS 2000).", making it non-representative for the general population. More recently styrene was biomonitored over a 2 year period in 150 disadvantaged minority children in Minneapolis Minnesota. The mean concentration was 0.12 µg/L and upper 95th percentile was 0.5 µg/L. In a follow-up study of 43 children aged 3 to 6 years and also from a disadvantaged neighborhood levels were lower and similar to those in the non-represented sample by the United States CDC.

Occupational exposure data collected in epidemiological studies are often of poor quality. Occupational exposure to styrene-7,8-oxide, for which there is sufficient evidence of carcinogenicity in animals, can occur directly, e.g. in "lamination". The extent of such exposure needs to be established.

Air concentrations measured in occupational settings where styrene is used in fabrication or manufacturing are orders of magnitude higher than environmental concentrations. This includes measurements reported in recent studies published since the IARC Monograph. In addition to characterizing exposures, biomonitoring studies continue to evaluate possible markers of styrene exposure. For example, Fustinoni et al. (2008) investigated "urinary analytes and haemoglobin and albumin adducts as biomarkers of exposure to airborne styrene (Sty) and styrene-(7,8)-oxide (StyOX) and to evaluate the influence of smoking habit and genetic polymorphism of metabolic enzymes GSTM1 and GSTT1 on these biomarkers." While protein adducts were not associated with styrene or styrene oxide exposures urinary metabolites were. Saturating metabolism was observed for all metabolites but for the mercapturic acids. The extent to which metabolic saturation in humans should be addressed in characterizing exposures in epidemiologic studies deserves greater attention.

Cancer in humans:

Styrene: limited, Vol 82; styrene oxide: inadequate, Vol. 60

Since the Monograph, two epidemiology studies were published (Ruder et al., 2004, Delzell et al., 2006 and Sathiakumar et al., 2009; Delzell et al., more completely reports on same cohort as Sathiakumar et al., 2005). The questions that remained open from the 2002 evaluation are:

- why was cancer risk concentrated in cohorts less exposed to styrene (in particular not in the reinforced plastics, where exposure levels are higher)
- the effect of potentially confounding exposures, in particular 1,3-butadiene
- why different types of hematolymphopoietic cancers are found in different cohorts
- whether cancers other than hematolymphopoietic (e.g. pancreatic) are increased in exposed workers.

The recent studies do not help address these questions in a conclusive manner. The study by Ruder is small and finds no excess of hematolymphopoietic cancers, while it finds a strong excess of kidney and bladder cancers. Like many cohort studies with information only from personnel records, it could not address properly the issue of multiple confounders. Sathiakumar et al. (update of Delzell) find a slight (16%) excess of leukemias in styrene-butadiene workers and again cannot rule out confounders. As noted in the NTP report, significant increases in leukemia were reported for this cohort with longer employment and latency, with highest cumulative styrene exposure. Also, relative risk increased with increasing cumulative styrene exposure, but was attenuated when controlled for butadiene, and disappeared when controlling for dimethyldithiocarbamate. Risk did increase with increasing exposure to styrene peaks, which persisted after controlling for butadiene and dimethyldithiocarbamate.

Cancer in experimental animals

Styrene: limited, Vol 82; styrene oxide: sufficient, Vol. 60

No new bioassays on styrene or styrene oxide have been published since the release of the IARC Monograph. In considering the need for further experimental evidence for evaluating animal carcinogenicity of styrene, three issues are noteworthy.

1) IARC updated the Preamble; the guidance for evaluating the evidence for carcinogenicity, after the 2002 styrene review. IARC updated the guidance on use of historical control data in evaluating tumor increases, e.g.,:

> "Less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls, particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals."

IARC also has updated the approach for evaluating a robust study conducted in both sexes:

> "An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*. "

2) Further statistical analyses of the available bioassay data have been conducted and are included in the report released by the NTP in 2008. The statistical significance of a variety of findings in the experimental animal studies is now clarified.

The 2008 expert NTP panel concluded that there was sufficient evidence of carcinogenic activity from multiple studies in mice by multiple routes.

A consistent finding across studies is the occurrence of lung neoplasia in mice. These are seen in a robust inhalation study in male and female CD-1 mice (Cruzan et al., 2001), and in male B6C3F1 mice exposed by oral intubation (NCI, 1979). The data in the intubation study was questioned because the incidence of lung tumors in the control males was thought to be low. The NTP reviewed the appropriate historical control data (animals from the same source, same study protocol and same chronological window, with similar duration) and found that the increases were significant. Furthermore, they were significant when compared with the concurrent controls. Early onset of lung tumors and increased incidence of lung tumors was also seen in male and female mice O_{20} mice receiving styrene by gavage, although the study had notable design limitations. It is also noteworthy that in the study of a mixture of styrene and beta-nitrostyrene lung tumors in mid-dose male mice were substantially higher than in controls (mortality in the top dose group was high, limiting the power to detect findings in this group).

Given the consistent finding of lung tumors and little else in the mouse, further long-term bioassays of traditional design in this species does not appear justified, even given IARC's label of limited evidence for animals. As discussed below, at the time of the IARC monograph and to present time, mechanisms by which styrene induces lung tumors in the mouse has been an active area of research.

3) The modulation of mammary tumors in a few rat studies (increased in three studies, and decreased in one study (Cruzan,) add justification for conducting research on potential carcinogenesis of styrene at tissues in addition to the lung, and in the mammary gland in particular, given the further discussion below. The lack of finding of mammary gland in another robust study in the same animal strain limited the weight given to the mammary tumor findings.

Styrene oxide consistently caused benign and malignant tumors of the forestomach, but not at other sites, following oral administration. Thus, tumorigenic effects have only been seen local to the site of administration, and not at distal sites. The localized impact of styrene oxide on cells, like the Clara cell, with have activities for metabolic transformation is the subject of research. Pharmacokinetics may in part explain the apparently different sensitivities and site specificities among rats, mice and humans but not necessarily on the cellular or microcompartment level. This deserves further study.

Mechanism of carcinogenicity

IARC did not upgrade or downgrade styrene, but as described above did upgrade styrene oxide, based on mechanistic data.

Styrene itself (unmetabolized) is a weak genotoxic agent. Studies in humans on chromosome aberrations are small and most are limited in design. However it is interesting to note that (a) styrene adducts have been found in lymphocytes of workers; (b) styrene is metabolized mainly to styrene-7,8,-oxide, which is genotoxic and is carcinogenic to animals with sufficient evidence; (c) the same DNA adducts have been found in humans and animals.

There are at least 70 research publications released since the styrene monograph that address various facets of the possible mechanisms of carcinogenic action in humans and rodents. Most of these have been reviewed in the NTP (2008) report and many by Vodicka et al. (2006). Briefly:

- A large number of studies address different aspects of human variability in enzymes involved in the metabolic activation and detoxification of styrene, some studying the impact on cytogenetic markers, DNA strand breaks and repair.
- Dose response relationships between DNA damage and styrene or styrene oxide exposure have been further explored.
- In addition to styrene-7,8-oxide, there has been some study of the possible role of other metabolites (e.g., styrene-3,4-epoxide and styrene-2,3-oxide), sometimes using 2E1 knockout mice.
- Related to the potential importance of extrahepatic metabolism, studies have recently been published that identify and quantify CYPs in a variety of human tissues, in addition to compartments of human lung.

- Induction of prolactin has been recently observed in humans, adding to the concern about possible mammary cancer risk. The vast majority of subjects in worker studies are men; women have not been studied to any significant degree.
- Some studies have explored the role of oxidative stress.
- There has been recent compartmental modeling of the pharmacokinetics in the form of physiologically based pharmacokinetic models, but validation of these models is in large part lacking.

The 2008 NTP review noted that at least two mechanisms of carcinogenesis were supported by the literature. First, it noted that the compound's genotoxicity to human lymphocytes involves sister chromatid exchange and chromosomal aberrations and that further that it involves the 7,8-oxide. It noted that CYP2F2 in mouse cells can efficiently metabolize the compound to this oxide, and that this enzyme and CYP2A13 and CYP2S1 that also have activity for styrene are expressed in various extrahepatic human organs and thus may bioactivate styrene. The panel also noted that the same adducts formed in vitro by incubating styrene oxide in culture also form in workers exposed to styrene.

The second mechanism noted is one discussed at length in the IARC review – the activation of the compound to an epoxide that is toxic to mouse lung cells by way of the formation of 4-vinylphenol and its further oxidation. The NTP panel noted that this may be operative in the human lung as well.

Priorities involve a further understanding of the detail of the chemicals pharmacokinetics. including the development and validation of PBPK models, and an expansion of studies on DNA adducts in humans and animals.

Research needs and recommendations

Research recommendations are:

1. to perform a pooled analysis of human studies on chromosome aberrations and other genotoxic effects (building on Bonassi et al. (1996))
2. to explore further extrahepatic metabolism of styrene by mammary, hematolymphopoietic, human lung and other tissue/cells
3. to study the formation of other possible genotoxic metabolites such as the 2,3- and 3,4- oxides, and their genotoxicity.
4. to compare DNA adduct formation in humans and animals
5. to refine estimates of the extent of internal exposure to styrene-7,8-oxide, and explore the possible magnitude of exposure to the cell by considering microcompartments or quantifying possible exposure at the cellular level.
6. to further explore and quantify the distribution of susceptibility to styrene based on interindividual differences in activation, detoxification enzymes and repair capacities.
7. to further examine the extent of human exposure to styrene-7,8-oxide, which has sufficient evidence of carcinogenicity in animals, e.g. in "lamination"

8. to update existing studies in humans, in particular those of reinforced plastics workers, addressing the open questions above and clarifying the issue of hematolymphopoietic cancer classification; also, a pooled analysis of these studies would be helpful
9. to recruit new cohorts
10. to promote nested case-control studies in the largest cohorts, particularly on hematolymphopoietic, bladder, kidney and pancreatic cancers, to look at: (a) levels of exposure and dose-response relationship; (b) potential confounders, e.g. 1,3-butadiene; (c) measures of biomarkers of long-term exposure, such as DNA or hemoglobin adducts.
11. to further explore the relationship between styrene exposure, potential increases in prolactin, and potential for mammary carcinogenesis by styrene since there are limited data on the elevation of prolactin in exposed workers, and since some of the animal studies produced suggestive evidence for this site.

Styrene is currently classified as Group 2B. The large body of evidence that has been generated since the last monograph together with the evidence in the monograph may support a re-evaluation of carcinogenicity. Some of the research recommendations above are to analyze and synthesize existing data. This could be useful in a reevaluation of the compound.

Selected relevant publications since IARC review

Fustinoni S, Campo L, Manini P, et al. An integrated approach to biomonitoring exposure to styrene and styrene-(7,8)-oxide using a repeated measurements sampling design. *Biomarkers* 2008; 13: 560-78.

Gokhale S, Kohajda T, Schlink U. Source apportionment of human personal exposure to volatile organic compounds in homes, offices and outdoors by chemical mass balance and genti algorithm receptor models. *Sci Tot Environ* 2008; 407: 122-138.

National Toxicology Program (NTP, 2008), Report on Carcinogens Background Document for Styrene, U.S. Department of Health and Human Services, NTP, Research Triangle Park, North Carolina, September, 29, 2008.

Ruder AM, Ward EM, Dong M, Okun AH, Davis-King K. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. *Am J Ind Med* 2004; 45: 165-176.

Sathiakumar N, Graff J, Macaluso M, et al. An updated study of mortality among North American synthetic rubber industry workers. *Occup Environ Med* 2005; 62: 822-829.

Vodicka P, Koskinen M, Naccarati A et al. Styrene metabolism, genotoxicity, and potential carcinogenicity. *Drug Metab Rev* 2006; 38: 805-853.