Critical Reviews in Toxicology, 24(S1):S117–S125 (1994)

Styrene: Toxicity Studies — What Do They Show?

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ABSTRACT: Styrene is efficiently metabolized to styrene oxide, which is itself readily detoxified by the same enzymes as those involved in the metabolism of various foodstuffs. Styrene oxide, like many intermediate metabolites of foodstuffs, is genotoxic and, if introduced directly into the stomachs of rodents in high doses/concentrations, gives rise to cancers of the forestomach. Exposing mice to doses of styrene high enough to overwhelm the capacity of the body to detoxify styrene oxide has been reported to increase lung tumor incidence in mice. The findings in eight epidemiological studies provide reassurance that occupational exposure to styrene is not associated with increased cancer risk. Tests for reproductive toxicity have given negative results, but effects on blood dopamine and hypothalamic and pituitary function and menstrual cycling under conditions of very high exposure have been reported. In light of all the available information, it is concluded that migration of styrene from food-wrapping materials is not a matter for toxicological concern.

KEY WORDS: styrene, styrene oxide, carcinogenicity, epidemiology, reproductive toxicity.

I. INTRODUCTION

Of the many ways in which toxicity may become manifest, three (mutagenicity, carcinogenicity, and effects on reproduction) generate the highest levels of concern.

I propose to devote most of the time available to me to discussing whether exposure to styrene, either at work or during everyday life, increases the risks of mutation or development of cancer. However, at the end of my talk I will say just a few words about the possible adverse effects of styrene on reproduction.

During the last few years, understanding with regard to the mechanisms involved in carcinogenesis has in many ways become more realistic. A widely accepted concept up to about 10 years ago may be summarized as follows: (1) all carcinogens are mutagens, (2) all mutagens are carcinogens, (3) any substance found to cause mutation or DNA damage in any test system, irrespective of its artificiality or the concentration or dose of the test substance, should be strongly suspected of carcinogenicity, and (4) in animal tests, a statistically significant increase in the incidence of any kind of benign or malignant tumor, irrespective of the dose, is indicative of carcinogenicity and a basis for regulatory disapproval.

Nowadays, there is widespread recognition that the following facts need to be taken into account:

1. Many chemicals that seemingly are not mutagens have been found to increase the risk of development of cancers in animals.
2. Many chemicals that have given positive results in tests for mutagenicity cannot be shown to increase cancer risk in animals.
3. A high dosage may give false positive results in animal tests because normal detoxification pathways are overwhelmed.
4. A high dosage may disturb the normal physiological status, particularly hormonal status, in such a way that the incidences of some kinds of tumor are either increased or decreased.
5. Many naturally occurring substances, including many in raw food and some formed during cooking, are mutagens.
6. Mutagens are formed normally in the body during the conversion of food to energy.

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7. DNA damage and repair of DNA damage occur in every cell in the body a great many times each day.
8. Overnutrition increases the disturbance of physiological status and dramatically increases the risk of development of most kinds of tumor. A slight dietary restriction substantially protects against this latter risk.

It is against this background of changing concepts of carcinogenesis mechanisms[21] that, in 1994, the safety of styrene needs to be reviewed.

II. METABOLISM AND DETOXIFICATION OF STYRENE

A great deal is known about the fate of styrene after it is taken into the body. The first thing that happens is that it is transformed to styrene oxide by enzymes present in the liver and other tissues (Figure 1). Styrene oxide is a reactive chemical, and its formation from styrene in the body has therefore been regarded as a matter for concern. However, the fact that the enzymes needed for its detoxification (Figure 2) are the same as those needed for the detoxification of numerous other chemicals, including many chemicals that are naturally present in food, means that there is nothing unique or alarming about the formation of low levels of styrene oxide in the body.

Theoretically, the picture might change if, because of high exposure to styrene, the capacity of the body to detoxify styrene oxide were to be exceeded. If this were to happen, then it might accumulate in the body or its breakdown might follow alternative metabolic routes involving a real risk of carcinogenicity. There is no evidence that the capacity for detoxification by the usual routes in fact happens, either in laboratory rats that have been exposed to very high dose levels of styrene or in humans exposed to atmospheres containing up to 100 ppm styrene, which is the highest limit set in national occupational standards. However, in mice, a sharp increase in blood styrene oxide levels occurs with exposure to styrene concentrations higher than 260 ppm, indicating that in this species, normal detoxification pathways for styrene can be overwhelmed.

III. MUTAGENICITY OF STYRENE AND STYRENE OXIDE

Styrene oxide is the sort of chemical that can react with DNA and bring about mutations. Its formation from styrene has therefore led to a fear

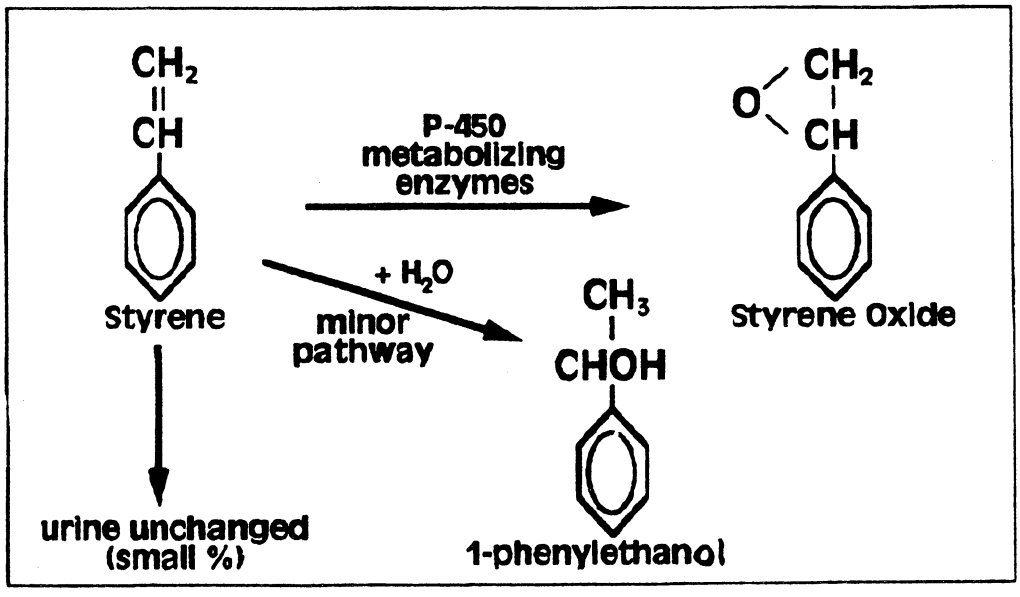

FIGURE 1. First step in the metabolism of styrene.

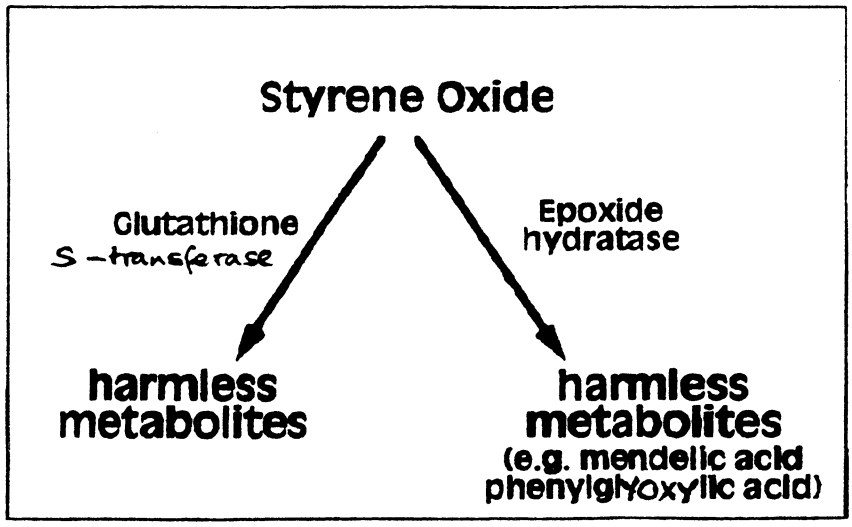

FIGURE 2. Detoxification of styrene oxide to form harmless chemicals that are secreted by the kidneys.

that exposure to styrene may increase the risk of cancer development. Furthermore, by themselves, the results of a wide variety of laboratory tests for mutagenicity have not proved that this concern is wholly unfounded. An overview of the available data indicates that styrene per se is not genotoxic in any way. However, in laboratory test systems involving the presence of P-450 enzymes capable of converting styrene to styrene oxide, positive results have been obtained. According to Phillips,[18] styrene oxide is only very weakly reactive with DNA. Whether or not mutation is a risk in styrene workers, however, is far from clear. Increased numbers of chromosomal aberrations, including sister chromatid exchanges, have been reported in the circulating lymphocytes of some styrene workers. However, this has not been a consistent finding. Furthermore, the small size of the studies and the impossibility of being sure that the effects were not due to exposure to other chemicals at work or to tobacco smoking, etc. leaves the interpretation of the findings in serious doubt.

IV. RESULTS OF ANIMAL TESTS ON STYRENE PER SE FOR CARCINOGENICITY

There have been eight carcinogenicity studies on styrene per se in rats and four in mice. All but three of these 12 studies involved exposure by the oral route. The other three were by inhalation. In addition, there have been three studies involving the oral administration of styrene oxide to rats and two studies involving its application to the skin of mice. The findings in 12 studies on styrene are summarized in Tables 1 and 2.

The only suggestively consistent positive finding in 12 studies on styrene per se was an increase in lung tumors in three of the four studies in mice. The enhancement of lymphoreticular neoplasia seen in the rat study reported by Jersey et al.[8] (see Table 3) is not clearly indicative of any real effect, particularly in view of the lack of any dose-related trend in males.

Overall, the results of these 12 studies in which rats or mice were heavily exposed to styrene per se gave results that were fairly convincingly negative for carcinogenic activity except for a possible marginal adverse effect on lung tumor incidence in mice exposed to dose levels that were within the toxic range wherein normal detoxification mechanisms are known to be exceeded.

V. RESULTS OF CARCINOGENICITY STUDIES ON STYRENE OXIDE

A priori, it was to be expected that exposure to styrene oxide might increase the risk of tumor

TABLE 1
Results of Eight Carcinogenicity Studies in Rats on Styrene

Ref.	Year	Route[a]	Highest dose	Result[b]
19	1978	GA	500 mg/kg/week	Negative (all sites)
14	1978	GA	700 mg/kg/d	Negative (all sites)
15	1979	GA	2000 mg/kg/d	Negative (all sites)
6	1988	GA	250 mg/kg/d	Negative (all sites)
1	1985	DW	250 ppm	Negative (all sites)
8	1978	IN	1000 ppm (30 h/week)	?? Mammary + ? Lymphoma/ leukemia
11	1982	IN	300 ppm (20 h/week)	Negative ++
6	1988	IN	300 ppm (20 h/week)	?? Mammary +

[a] GA, gavage; DW, drinking water; IN, inhalation.
[b] +, not dose related; ++, only brain examined.

TABLE 2
Results of Four Carcinogenicity Studies in Mice on Styrene Given by Gavage

Ref.	Year	Dose	Result
19	1978	1350 mg/kg, 1/week (16 weeks)	Lung tumors
19	1978	300 mg/kg, 1/week (120 weeks)	No significant effect
15	1979	300 mg/kg, 5/week (78 weeks)	Lung tumors
14	1978	407 mg/kg, 3/week (78 weeks)	No significant effect

TABLE 3
Incidence of Leukemia/Lymphosarcoma Observed by Jersey et al. (1978) in Sprague-Dawley Rats Exposed to Styrene by Inhalation

Dose level (ppm)	Males	Females
0	1/85	1/85
600	5/84	6/85
1200/1000	1/86	6/85

development in tissues that come directly into contact with it. Thus, it is not surprising that in both rats and mice the repeated administration by gavage of very high doses of styrene oxide led to the development of tumors of the forestomach, which was the first tissue with which the administered dose came into contact (see Tables 4 and 5). It is probable that exposure to lower doses/concentrations of styrene oxide would have no effect on the incidence of forestomach tumors in ro-

TABLE 4
Results of Four Carcinogenicity Studies in Rats on Styrene Oxide

Ref.	Year	Route	Dose	Result
10	1979	Gavage	250 mg/kg, 4–5/week (52 weeks)	Negative except for forestomach tumors
6	1988	Gavage	250 mg/kg, 4–5/week (52 weeks)	Negative except for forestomach tumors
20	1984	Gavage	150 mg/kg, 1/week (96 weeks)	Negative except for forestomach tumors
9	1986	Gavage	500 mg/kg, 3/week (104 weeks)	Negative except for forestomach tumors

TABLE 5
Results of Three Carcinogenicity Studies in Mice on Styrene Oxide

Ref.	Year	Route	Dose	Result
23	1963	Skin	10% 3/week (life)	Negative
22	1963	Skin	10% 3/week (life)	Negative
9	1986	Gavage	750 mg/kg (104 weeks)	Negative except for forestomach tumors

dents. However, no studies involving exposure to lower doses have been reported. In any case, in my opinion, the most significant aspect of the findings was that in neither species was there any evidence of a carcinogenic effect at any other site. This strongly suggests that the detoxification of styrene oxide is a very efficient process.

VI. RESULTS OF STUDIES ON WORKERS EXPOSED TO STYRENE

There has been a total of eight epidemiological studies of persons exposed occupationally to styrene (Table 6). Between them, they have involved the follow-up of about 50,000 persons

TABLE 6
Brief Details of Eight Epidemiological Studies

Ref.	Period	Years	% traced	Deaths
2	40–86	47	99	687
12	43–79	37	93	1995
7	45–78	34	97	622
13	43–76	34	97	332
16	60–75	16	100	83
5	47–84	38	97	693
17	57–78	22	96	176
24	48–77	30	84	499

over periods of up to 47 years. A total of 5087 death certificates are available for analysis. Five of the eight studies (>20,000 persons) concern workers exposed to styrene during its manufacture and use in polymer production and/or its use in the production of styrene-butadiene latex copolymers (Table 7). The other three studies concern workers involved in the manufacture of reinforced plastics. The ambient air levels to which workers in the latter three studies were exposed were 50 to 100 times higher than that to which those in the first five studies were exposed. In seven of the eight studies, the numbers of deaths from all causes were substantially less than the numbers expected based on figures for the populations from which the workers stemmed (Table 8). However, this does not indicate that exposure to styrene confers health benefits. It simply reflects that the health of people capable of working is on average better than that of populations that include people incapable of working. This phenomenon is commonly referred to as the "healthy worker effect". Mortality from any form of cancer also was lower than expected in seven of the eight studies (Table 9). Deaths from circulatory diseases, respiratory diseases other than cancer, and diseases of the digestive system, including gut cancers, were fewer than expected in all eight studies.

TABLE 7
Industries Represented and Extent of Exposure to Styrene in Eight Epidemiological Studies

	Styrene monomer and polymer production	Styrene-butadiene latex co-polymer production	Reinforced plastics manufacture
Typical ambient levels of styrene (ppm)	**1**	**1**	**50–100**
Ref 2	+	+	
Ref. 12		+	
Ref. 7	+		
Ref. 13		+	
Ref. 16	+	+	
Ref. 5			+
Ref. 17			+
Ref. 24			+

TABLE 8
Mortality from Major Causes as Percent of Expected

Ref.	All causes	All cancers	Circulatory diseases	Respiratory diseases except cancer	Diseases of digestive system
2	76	81	83	55	63
12	81	84	79	64	48
7	80	90	90	49	—
13	77	72	87	71	67
16	78	81	92	15	—
5	83	81	89	68	99
17	90	103	72	82	79
24	100	88	93	52	76

TABLE 9
Mortality from Cancers of the Gut, Lung and Haemopoietic System in the Eight Studies as a Percent of Expected

Ref.	Gut cancer	Respiratory cancer	Leukemia, lymphoma, etc.
2	77	80 (58 vs. 72.5)	144 (28 vs. 19.5)
12	97	85 (134 vs. 157.6)	85 (40 vs. 47.1)
7	—	119 (5 vs. 4.2)	349 (3 vs. 0.9)[a]
13	58	85 (21 vs. 24.6)	132 (11 vs. 8.3)
16	—	85 (6 vs. 7.0)	98 (2 vs. 2.0)
5	65	111 (89 vs. 80.1)	40 (6 vs. 14.9)
17	95	143 (16 vs. 11.2)	0 (0 vs. 4.2)
24	89	116 (34 vs. 29.3)	73 (9 vs. 12.3)

[a] Includes two cases that led to the study being undertaken.

In four of the five studies of workers in the occupations with only low levels of exposure to styrene, respiratory tract cancers caused fewer deaths than expected. In the fifth of these studies five deaths were observed compared with only 4.2 expected —clearly a trivial difference that can be attributed to chance. By contrast, in all three studies on the more heavily exposed workers involved in reinforced plastics manufacture, the mortality from respiratory cancers was higher than expected. A closer look at the data, however, shows that in the Okun study,[17] eight of the 16 workers who died of respiratory cancers had worked in the industry for less than 6 months. Also, in the Wong study,[24] there was an inverse relationship between respiratory cancer mortality and length of exposure to styrene.

These inverse relationships with length of exposure, combined with the lack of data about the smoking habits of those who died from respiratory tract cancers, render it most unlikely that exposure to styrene adversely affects the risk of death from respiratory tract cancers.

Finally, we need to look at the data with respect to death from leukemia/lymphoma, etc. The first thing to note is that far fewer deaths than expected were seen in the three studies involving the higher exposure to styrene. This fact alone makes it difficult to believe that exposure to styrene was responsible for the higher than expected incidence of leukemia/lymphoma seen in the five studies on workers exposed to much lower levels of styrene. Furthermore, there are, again, cogent reasons for doubting the role of exposure to styrene per se in the causation of the cancers in these cases. (1) The study by Hodgson[7] was, in fact, undertaken because two cases of lymphoma/leukemia had been observed; thereafter, the follow-up of a total of 622 workers revealed only one more case vs. 0.9 cases expected. (2) In the three studies in which a higher than expected incidence was seen, there is no indication of any especially high risk of any particular form of lymphoreticular cancer (e.g., Hodgkin's lymphoma, non-Hodgkin's lymphoma, myeloma, leukemia). (3) The data from the largest study[12] showed no evidence of increased risk. (4) In the Bond study,[2] there was no supportive evidence of increased risk associated with either duration of exposure or level of exposure.

My colleague, Peter Lee, who as a professional epidemiologist/statistician carefully reviewed the data, concluded:

1. The evidence that styrene increases the incidence of cancer of lymphatic and hemoietic tissue is very weak indeed.
2. My general impression is that the apparent increase in risk in styrene monomer and polymer producers, if not due to chance, probably is due to other chemicals to which the workers were exposed.

VII. REPRODUCTIVE TOXICITY (TABLE 10)

Brown[3] reviewed the available data from laboratory studies and observations on humans with respect to the possible reproductive and developmental toxicity of styrene. He concluded that, in response to exposure levels that do not cause maternal toxicity but which are very much higher than those to which humans are exposed occupationally, styrene is not teratogenic, is not fetotoxic, does not increase perinatal mortality, and does not adversely affect fertility in either sex. On the other hand, there is some evidence that, under conditions of high exposure, styrene can affect blood dopamine and consequently hypothalamic and pituitary function. These effects may possibly disturb estrous cycling in animals and, possibly, menstrual cycling in women. Such disturbances do not occur under realistic levels of exposure.

TABLE 10
Reproductive Toxicology of Styrene

No evidence of teratogenicity
No evidence of fetotoxicity
No increased perinatal mortality in absence of maternal toxicity
No adverse effect on fertility
? High dose effect on brain dopamine might affect hypothalamus and pituitary (disturbed estrous cycling in animals, menstrual cycling in women)

From Brown, N. A., *Reprod. Toxicol.*, 5, 3, 1991. With permission.

VIII. OVERALL CONCLUSIONS

1. If styrene is absorbed into the body, it is metabolized to styrene oxide.
2. Styrene oxide is readily detoxified by the enzymes responsible for detoxifying a wide variety of other chemicals, including many that occur plentifully in food or which are formed during the metabolism of foodstuffs.
3. The capacity of this detoxification pathway considerably exceeds the requirement for it under the conditions in which workers are exposed to styrene.
4. Styrene oxide, like many metabolites of foodstuffs, is a reactive chemical that can damage DNA and give rise to genotoxic effects. High doses/concentrations of styrene oxide introduced directly into the stomachs of rodents predispose to the development of cancers in a part of the stomach (forestomach) that humans do not have.
5. A total of 12 studies involving the long-term exposure of rats and mice to styrene by either the oral route or inhalation have given no convincing evidence of carcinogenicity except possibly in mice exposed to very high doses that overwhelmed the capacity for detoxification of styrene oxide. In two out of four such studies, the incidence of lung tumors was increased. However, the effect was no more striking than the difference in the incidence of lung tumors between *ad libitum*-fed mice and mice fed 80% of *ad libitum* of the same standard laboratory chow.
6. The findings in eight epidemiological studies have provided substantial reassurance that occupational exposure to styrene does not increase the risk of cancer generally or of cancers of the respiratory, digestive, or blood-forming tissues in particular.
7. *A fortiori,* there is absolutely no basis for concern that nonoccupational exposure to styrene, as may occur as a consequence of migration from food wrapping materials or through contact with styrene-based polymers generally, might increase the risks of developing any form of cancer.

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