FINAL

Report on Carcinogens Background Document for

Styrene-7,8-oxide

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

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

US Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

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 Carcinogens:

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 a *known to be human carcinogen, or reasonably anticipated to be 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.

Summary Statement

Styrene-7,8-oxide

CASRN 96-09-3

Carcinogenicity

Styrene-7,8-oxide (1,2-epoxyethylbenzene, styrene epoxide, 96-09-3) is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenic activity at multiple tissue sites in multiple species of experimental animals. Styrene-7,8-oxide given by oral intubation induced high incidences of both benign and malignant tumors of the forestomach in both sexes of rats (three strains) and mice (one strain) (Maltoni *et al*. 1979, Ponomarkov *et al*. 1984, Lijinsky 1986, Conti *et al*. 1988, all cited in IARC 1994a). Additionally, tumors of the liver were increased in exposed male mice (Lijinsky 1986).

There were no case reports or epidemiological studies of the occurrence of human cancer and exposure to styrene-7,8-oxide.

Other Information Relating to Carcinogensis or Possible Mechanisms of Carcinogenesis

Styrene-7,8-oxide is genotoxic in a variety of prokaryotic, plant, eukaryotic, and mammalian (including human) *in vitro* and *in vivo* systems. Styrene-7,8-oxide induces mutations in bacteria, yeast, insects, and cultured mammalian cells and clastogenic activity (chromosomal aberrations or sister chromatid exchanges) in Chinese hamster V79 cells, Chinese hamster ovary cells, mouse bone marrow cells *in vivo*, and cultured human lymphocytes. Styrene-7,8-oxide induced mutations at the *hprt* locus in Chinese hamster V79 cells and in human T lymphocytes. DNA strand breaks occurred after treatment with styrene-7,8-oxide of cultured primary animal hepatocytes, human embryonal cells, and human lymphocytes and in lymphocytes, liver, and kidney cells in mice. DNA adducts were formed in several organs in mice and in cultured mammalian cells. A study of workers in a boat-making facility, where styrene concentrations ranged from 1 to 235 mg/m^3 (mean of 65.6 mg/m^3, or 13.3 ppm), reported an increase in styrene-7,8-oxide DNA adducts in mononuclear cells. DNA adducts in rodents and humans appear to be similar.

Styrene-7,8-oxide is absorbed by rabbits, rats, and mice following oral administration and hydrolyzed rapidly in the acid environment of the stomach. Almost all of the absorbed dose is excreted in the urine of experimental animals. Styrene-7,8-oxide can be metabolized by epoxide hydrolase to the glycol or by glutathione S-transferase to glutathione conjugates. Styrene glycol is further metabolized to mandelic, phenyl glyoxylic, and hippuric acids, which are excreted in urine.

Urine of workers exposed to styrene-7,8-oxide vapors contained large amounts of mandelic acid and phenylglyoxylic acid, both known metabolites of styrene-7,8-oxide. DNA and albumin adducts were found in blood of plastics workers exposed to styrene-7,8-oxide. Low levels of

covalent binding of styrene-7,8-oxide to DNA adducts were observed in the stomachs of orally dosed rats.

No data are available that would suggest that mechanisms thought to account for genotoxic effects and tumor induction by styrene-7,8-oxide in experimental animals would not also operate in humans.

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1 Introduction

Styrene-7,8-oxide (SO) was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS) Report on Carcinogens Review Group (RG1) based on review of an International Agency for Research on Cancer (IARC) monograph (IARC 1994) which indicated sufficient evidence for the carcinogenicity of SO in experimental animals and that it is *probably carcinogenic to humans* (Group 2A).

1.1 Chemical identification

SO (C_8H_8O, mol wt 120.15, CASRN 96-09-3) is a colorless to pale straw-colored liquid and is also known by the following names:

styrene oxide	1-phenyl-1,2-epoxyethane
styrene epoxide	phenyloxirane
1,2-epoxyethylbenzene	epoxyethylbenzene
epoxystyrene	alpha, beta-epoxystyrene
phenethylene oxide	phenylethylene oxide
2-phenyloxirane	styryl oxide
phenyloxirane, d8	styrene oxide-d8.

The RTECS number for SO is CZ9625000.

1.2 Physical and chemical properties

The structure of SO is illustrated in Figure 1-1, and its physical and chemical properties are summarized in Table 1-1. SO is a corrosive chemical that reacts vigorously with compounds having labile hydrogen, including water, and in the presence of catalysts such as acids, bases, and certain salts. It polymerizes exothermically (Clayton and Clayton 1981, cited in HSDB 1994a). It is soluble in benzene, acetone, methanol, carbon tetrachloride, and heptane (IARC 1994a).

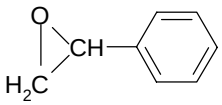

Figure 1-1. Structure of SO

Source: Chemfinder (1999)

Table 1-1. Physical and chemical properties of SO

Property	Information	Reference
Molecular weight	120.15	CRC (1993)
Color	colorless to straw-colored	Sax and Lewis (1987)
Odor	sweet, pleasant	Verschueren (1983)
Physical state	liquid	IARC (1994a)
Melting point (ºC)	- 36.7	IARC (1994a)
Boiling point (ºC)	194.1	IARC (1994a)
Specific gravity at 16ºC/4ºC	1.0523	CRC (1993)
Density at 20°C/4°C	1.050 – 1.054	IARC (1994a)
Solubility:		
Water at 25°C	0.28%	Clayton and Clayton (1981)
Alcohol	soluble	CRC (1993)
Ether	soluble	CRC (1993)
Partition coefficient		
Log octanol water (Log P)	1.61	Hansch and Leo (1987)
Relative vapor density (air = 1)	4.30	Clayton and Clayton (1981)
Vapor pressure (mm Hg at 20°C)	0.3	IARC (1979)
Flash Point, (°C)	74	NFPG (1991)

1.3 Identification of metabolites

In mammals, SO is a major metabolite of styrene. Styrene undergoes oxidation by the microsomal monooxygenase system to SO, followed by rapid enzymatic hydration to styrene glycol or conjugation with glutathione (Harkonen 1978, cited in HSDB 1994b). Styrene glycol is oxidized to mandelic acid, which is further oxidized to phenylglyoxylic acid. The main metabolic end products of styrene in humans are mandelic and phenylglyoxylic acid (Leibman 1975, cited in HSDB 1994b). Styrene (C_8H_8, mol wt 104.15, CASRN 100-42-5) also is known by the following names:

phenylethylene
ethenylbenzene
styrolene
cinnamol
cinnamenol
phenethylene
styron
styropor
styrene monomer.
styrol
annamene
cinnamene
vinyl benzene
diarex hf 77
phenylethene
styropol
vinylbenzol

Styrene is a colorless liquid with a sweet, aromatic odor at low concentrations and a sharp penetrating odor at high levels. It is sensitive to light and air. The physical and chemical properties of styrene are summarized in Table 1-2. Styrene is a flammable liquid

(shipping code UN 2055). Its RTECS number is WL 3675000, and its structure is illustrated in Figure 1-2.

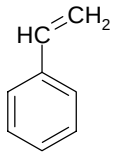

Figure 1-2. Structure of styrene

Table 1-2. Physical and chemical properties of styrene

Property	Information	Reference
Molecular weight	104.15	CRC (1993)
Color	colorless to yellowish oily liquid	NIOSH (1984)
Odor	sweet, floral odor	NIOSH (1984)
Physical state	liquid	NIOSH (1984)
Melting point (ºC)	- 30.6	CRC (1993)
Boiling point (ºC)	145.2	CRC (1993)
Specific gravity at 16ºC/4ºC	0.9045	Chemfinder (1999)
Density at 20ºC/4ºC	0.9060	CRC (1993)
Solubility:		
Water at 25°C	sparingly	IARC (1994a)
Alcohol	soluble	CRC (1993)
Ethanol	soluble	CRC (1993)
Acetone	soluble	CRC (1993)
Benzene	soluble	CRC (1993)
Partition coefficient		
Log octanol water (Log P)	2.95	Hansch and Leo (1987)
Relative vapor density (air = 1)	3.6	Chemfinder (1999)
Vapor pressure (mm Hg at 20°C)	6.12	HSDB (1994b)
Flash point (°C)	32	NFPG (1991)

SO is hydrolyzed *in vitro* to styrene glycol by microsomal epoxide hydrolase from the liver, kidneys, intestine, lungs, and skin of several mammalian species (Oesch 1973, cited in IARC 1985). The structure of styrene glycol ($C_8H_{10}O_2$, mol wt 138.17, CASRN 25779-13-9) is presented in Figure 1-3.

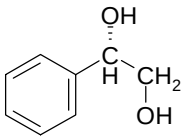

Figure 1-3. Structure of styrene glycol

Source: Chemfinder (1999)

Styrene glycol can be further metabolized to mandelic acid and benzoic acid (Vainio *et al.* 1984, cited in IARC 1985). The structure of mandelic acid ($C_8H_8O_3$, mol wt 152.15, CASRN 90-64-2) is presented in Figure 1-4.

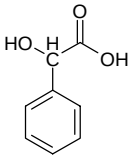

Figure 1-4. Structure of mandelic acid

Source: Chemfinder (1999)

Mandelic acid can be further metabolized to benzoic acid and phenylglyoxalic acid. Benzoic acid ($C_7H_6O_2$, Mol. wt. 122.12, CASRN 65-85-0) is a white powder used as a flavoring preservative. Its structure is illustrated in Figure 1-5. The structure of phenylglyoxylic acid ($C_8H_6O_3$, mol wt 150.13, CASRN 611-73-4) (Vainio *et al.* 1984, cited in IARC 1985) is illustrated in Figure 1-6.

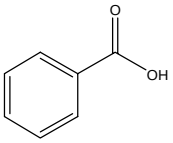

Figure 1-5. Structure of benzoic acid

Source: Chemfinder (1999)

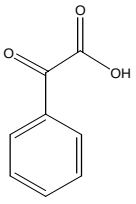

Figure 1-6. Structure of phenylglyoxylic acid

Source: Chemfinder (1999)

Benzoic acid can also be metabolized to hippuric acid ($C_9H_9NO_3$, mol wt 179.18, CASRN 495-69-2) (Vainio *et al.* 1984, cited in IARC 1985). The structure of hippuric acid is presented in Figure 1-7.

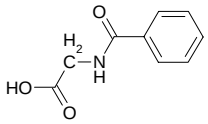

Figure 1-7. Structure of hippuric acid

Source: Chemfinder (1999)

2 Human Exposure

2.1 Use

SO is used mainly as an intermediate in the production of styrene glycol and its derivatives. It also is used as a reactive diluent for epoxy resins and as a chemical intermediate for cosmetics, surface coatings, and agricultural and biological chemicals. SO has been used as raw material for the production of phenylethyl alcohol, used in perfumes and in the treatment of fibers and textiles. SO's major use is in the production of reinforced plastics and in boat making (HSDB 1994a; U.S. EPA 1998).

2.2 Production

The U.S. International Trade Commission (U.S. ITC 1994) has no data on domestic SO production values for 1992. The Toxic Release Inventory (TRI 1996) identified five companies that produce, handle (by way of by-product), or manufacture SO in the United States. The U.S. Environmental Protection Agency (EPA) listed SO in its high production value chemical list, with SO production values from .75 to 1.28 million lb/yr (340,000 to 580,000 kg/yr) (U.S. EPA 1990).

2.3 Analysis

Siethoff *et al.* (1999) used inductively coupled plasma (ICP), high-resolution mass spectrometry and electrospray ionization mass spectrometry (MS), both interfaced to reversed-phase high-performance liquid chromatography (HPLC), to determine levels of DNA adducts in humans. With LC/ICP-MS, the detection of limit for SO adducts was determined to be 20 pg absolute or 14 modified/10^8 unmodified nucleotides in a 5-µg sample of DNA.

Reported methods for analysis of SO are summarized in Table 2-1 (IARC 1985).

Table 2-1. Methods for the analysis of SO

Sample matrix	Sample preparations	Assay procedure[a]	Limit of detection	Reference
Ambient air	collect on sorbent, desorb thermally	GC/MS	2 ng/m^3	Pellizzari *et al.*(1976), Krost *et al.* (1982)
Workplace air	collect on sorbent, extract (ethyl acetate)	GC/FID	0.2 ng in extract (0. 1 µg/sample)	Stampfer and Hermes (1981)
	collect on charcoal, extract (dichloromethane)	GC/FID; GC/MS	not given	Pfaffli *et al.* (1979)
Drinking water	concentrate, extract (ethanol), react with 4-nitrothiophenol	HPLC/UV	not given	Cheh and Carlson (1981)
Biological media	form picrate	GC/FID or TLC	not given	Leibman and Ortiz (1970)
Mouse blood	extract (dichloromethane), use *para*-methylanisole as an internal standard	GC/FID or GC/MS	10 ng/mL	Bidoli *et al.* (1980)

Sample matrix	Sample preparations	Assay procedure[a]	Limit of detection	Reference
Rat-liver homogenate	react with nicotinamide, incubate	fluorimetry	24 - 60 ng	Nelis and Sinsheimer (1981)
Commercial styrene chlorohydrin		TLC/spectro-photomtery	1.5 µg	Dolgopolov and Lishcheta (1971)
Aqueous solution	react with periodate, react with cadmium iodide-starch	spectro-photometry	not given	Mishmash and Meloan (1972)
	react with sodium sulphite	titration	not given	Swan (1954)
Acetone solution	reaction with 4-(*p*-nitrobenzyl)-pyridine/triethylamine	spectro-photometry	12 µg max	Agarwal *et al.* (1979)

[a]GC/MS, gas chromatography/mass spectrometry; GC/FID, gas chromatography/flame ionization detection; HPLC/UV, high-performance liquid chromatography/ultraviolet absorbance detection; TLC, thin-layer chromatography.

2.4 Environmental occurrence

SO does not naturally occur in the environment (IARC 1994a). It may enter the environment through industrial discharges or spills in wastewater, or through emissions (U.S. EPA 1998). SO also has been found as an impurity in commercial samples of styrene chlorohydrin (IARC 1985).

2.4.1 Air

In 1976, SO was identified in air samples collected in the Los Angeles Basin along and other unidentified areas in the United States. Quantitative amounts, however, were not reported (IARC 1985). Annual air emissions of SO in the United States were reported in 1987 as 464 kg (1,023 lb) from two locations, in 1988 as 1,050 kg (2,315 lb) from six locations, and in 1991 as 760 kg (1,676 lb) from five locations. Total releases to ambient water in 1987 were estimated at 353 kg (778 lb) (IARC 1994a). The Toxic Release Inventory (TRI) reported total releases of SO into ambient air in 1996 as 31 lb (14 kg) from four facilities (TRI 1996).

2.4.2 Water and sediments

In a comprehensive survey of 4,000 samples of wastewater taken from both industrial and publicly owned treatment centers in the United States, SO was found in one site. Discharge effluent from a rubber processing industry was identified as having a SO level of 46.2 ppb (µg/L) (IARC 1994a). SO also was identified in the effluent from a latex manufacturing plant in Louisville, KY, and chemical manufacturing plants in Louisville and Memphis, TN, but levels were not given (HSDB 1994a).

2.5 Environmental fate

When released into the environment, around 95% of SO will eventually be deposited in water, while the rest will be dispersed in the atmosphere (TRIFacts 1989).

2.5.1 Terrestrial fate

When released into soil, SO will leach into the ground, where it will rapidly degrade, because epoxides readily react with compounds containing active hydrogen groups. Degradation of SO in acidic soils will be faster, as hydrolysis is faster in acidic media (HSDB 1994a).

2.5.2 Aquatic fate

When released into neutral water, SO will hydrolyze with a half-life of 28 h. Hydrolysis would be faster in water with a lower pH. SO also will be lost by volatilization (half-life of 25 h in a model river), but this process will be competitive with hydrolysis only in rivers (HSDB 1994a). SO is highly water soluble (3,000 mg/L) and, therefore, will not bioaccumulate significantly in aquatic organisms (HSDB 1994a).

2.5.3 Atmospheric fate

When released to the atmosphere, SO will react with photochemically produced hydroxyl radicals. SO's estimated half-life in the atmosphere is 3.1 days (HSDB 1994a).

2.6 Environmental exposure

Exposure to the general population may occur as a result of contact with contaminated air or water. No data quantifying exposure were located.

Philo *et al.* (1997) analyzed various plastics and resins in the United Kingdom to determine whether SO could migrate to food. SO was found in 9 base resins and 16 samples of polystyrene articles that come into contact with food. Concentrations of SO in typical polystyrene materials were low, ranging from undetectable (< 0.5 mg/kg) to 3 mg/kg. Assuming that SO will migrate in the same pattern as the styrene monomer, estimates of migration to food range from 0.002 to 0.15 μg/kg (Philo *et al.* 1997).

2.7 Occupational exposure

Occupational exposure to SO occurs mostly in workers in the paints and allied products industry (NOHS 1981). The National Occupational Exposure Survey (NIOSH 1990) indicated that 457 employees were potentially exposed to SO in the United States between 1980 and 1983, of which it was estimated that 59% were exposed to SO and 41% to materials containing SO. SO is formed *in situ* at low levels in air (< 1 mg/m^3, < 203 ppb) when styrene reacts with oxygen or hydroperoxides (used to initiate the curing of reinforced plastics) (Yeowell-O'Connell *et al.* 1996).

The primary occupational exposure to SO is indirect and the result of exposure to styrene. Information concerning occupational exposures to styrene is provided for this reason. The National Occupational Exposure Survey (NIOSH 1990) determined that 108,000 workers, including 39,400 females, were exposed to styrene between 1982 and 1983.

In a boat-manufacturing company in the United States, the mean airborne SO exposure level was found to be 0.14 mg/m^3 (28.5 ppb) for 19 workers who also were heavily exposed to styrene (mean concentration 64 mg/m^3) (Rappaport *et al.* 1991, cited in IARC 1994a).

Rappaport *et al.* (1996) investigated various biomarkers to determine occupational exposure to SO. The mean exposure for 20 workers in a factory where boats were manufactured was 159 $\pm$ 25 $\mu g/m^3$ (32.4 $\pm$ 5.1 ppb). The range of exposure was 13.4 to 256 $\mu g/m^3$ (2.73 to 52.1 ppb). SO exposure for various occupations is shown in Table 2-2.

Table 2-2. Occupational exposure to SO

Job title	Number of subjects	Mean SO exposure ($\mu g/m^3$)	Mean SO exposure (ppb)
Laminator (including laminator supervisors)	11	182	37.0
Service	2	77.6	15.8
Mold repair	3	198	40.3
Patcher	2	96.0	19.5
Painter	1	158	32.2
Spray operator	1	74.4	15.1

Source: Rappaport *et al.* (1996)

2.7.1 Occupational exposure outside the United States

Nylander-French *et al.* (1999) studied workers who manufactured reinforced plastics to determine levels of SO exposure and possible factors contributing to SO exposure. In laboratory experiments, SO formation was postulated to occur from one of the following: fragmentation of polymeric styrene peroxide radicals resulting from the copolymerization of styrene and oxygen, epoxidation of the styrene monomer, or reaction of styrene with volatile organic peroxides used to initiate the curing of reinforced plastics. No field assessments have been able to confirm these speculations, however. Overall, SO exposure levels were positively correlated with styrene exposure levels. This correlation, however, was significant only among those workers with the highest levels of styrene and SO exposure, hand laminators. Resin use also was an important factor in predicting SO exposure, while quantity of the resin was not important. This study shows that factors other than styrene exposure obviously affect SO exposure levels (Nylander-French *et al.* 1999).

2.8 Biological indices of exposure

The main human urinary excretion products of styrene include phenylglyoxylic acid and mandelic acid, production of both of which indicates that SO is formed as an intermediate. Low concentrations of SO (0.05 $\mu g/L$) were detected in the urine of four workers who were exposed to styrene of unspecified purity (IARC 1985).

Studies dealing with styrene and SO exposure often have focused on styrene levels, because of the high levels present in certain occupations and because styrene has been shown to be metabolized to SO in humans. Recent studies have shown that while styrene is metabolized to SO via hepatic cytochrome P-450 isozymes, it is subsequently metabolized in the liver by epoxide hydrolase to form styrene glycol and its oxidation products phenylglyoxylic acid and mandelic acid. Thus, only a small proportion of the

styrene will remain as SO in the human body. The small amount of SO exposure is much more important, as the inhaled SO is absorbed into the blood, where it can react with macromolecules such as hemoglobin, albumin, and DNA. Calculations suggest that only ½,000 of an oral dose of styrene taken by humans would be found as SO in the bloodstream (Rappaport *et al.* 1996).

Yeowell-O'Connell *et al*. (1996) conducted research affirming the use of hemoglobin and albumin adducts as biomarkers of exposure to styrene and SO. Cysteine and carboxylic acid adducts of SO with hemoglobin and albumin were measured in 48 workers (both male and female). Analysis of carboxylic acid adducts, however, was not meaningful, because these adducts were not stable. GC-MS analysis indicated no exposure-related increase in hemoglobin adducts, whereas albumin adducts did increase with occupational SO exposure. Yeowell-O'Connell *et al.* (1996) also found that SO adducts of albumin were strongly correlated to SO exposure but not with styrene exposure. SO adducts of albumin and hemoglobin were detected in people who were not occupationally exposed to styrene or SO. This may indicate that SO is a dietary or environmental contaminant or is produced endogenously (Yeowell-O'Connell *et al.* 1996).

Fustinoni *et al.* (1998) compared levels of the SO urinary metabolites mandelic acid and phenylglyoxylic acid and SO adducts with hemoglobin and albumin. The group studied 22 male workers in Italy exposed to an undetermined amount of styrene in the reinforced-plastics industry. Urinary metabolites were analyzed by HPLC, and adducts were analyzed by GC-MS. The estimated mean levels of mandelic acid and mandelic acid plus phenylglyoxylic acid were 74 and 159 mg/g creatinine, respectively. Based on these means, the average workplace air concentration for styrene was estimated at about 100 mg/m^3 (20 ppm) for an Italian reinforced-plastics plant. Based on the data, only exposures to high levels of styrene allowed for a clear relationship between styrene exposure and SO adduct formation, because of the effects of cigarette consumption and high levels of SO adducts observed in unexposed subjects (Fustinoni *et al.* 1998).

2.9 Regulations

U.S. EPA regulates SO under the Clean Air Act (CAA) as a volatile hazardous air pollutant. SO also is regulated by U.S. EPA under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Superfund Amendments and Reauthorization Act (SARA). U.S. EPA regulations are summarized in Table 2-3. SO is regulated by the U.S. Food and Drug Administration (FDA) for use as a coating for certain containers. FDA regulations are presented in Table 2-4. The Occupational Safety and Health Administration (OSHA) does not regulate SO.

Table 2-3. U.S. EPA Regulations

U.S. EPA Regulations	
Regulatory action	Effect of regulation and other comments

U.S. EPA Regulations	
Regulatory action	Effect of regulation and other comments
40 CFR 63 – PART 63 – NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Codes: 7401 et seq.; CAA.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
40 CFR 63.680ff. – Subpart DD – Applicability and designation of affected sources. Promulgated: 61 FR 34158, 07/01/96. Styrene oxide is classified as a Hazardous Air Pollutant (HAP).	The provisions of this subpart apply to plant sites at which a major source of HAP Emissions occurs as defined in 40 CFR 63.2, or at which is located one or more operations that receives offsite materials as specified in 40 CFR 63.680(b).
40 CFR 63.800ff. – Subpart JJ – National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/07/95.	The provisions of this subpart apply to each facility that is engaged in the manufacture of wood furniture or wood furniture components and that is a major source as defined in 40 CFR 63.2. Styrene oxide is classified as a volatile HAP and is prohibited from use in cleaning and wash-off solvents.
40 CFR 172 – SUBPART B – Table of Hazardous Materials and Special Provisions. Promulgated: 61 FR 50623, 50624, 09/26/96.	The Hazardous Materials Table in this section designates SO as hazardous materials for the purpose of transportation of those materials. The reportable quantity for SO is 100 lb (45.4 kg).
40 CFR 302 – Part 302 – DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Codes: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities (RQ) for hazardous substances designated under section 311(b)(2)(A) of the CWA. The RQ for SO is 100 lb (45.4 kg).
40 CFR 372 – PART 372 – TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Codes: 42 U.S.C. 11013, 11028.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, to aid in the development of regulations, guidelines, and standards. The effective date for reporting releases of SO is 1/1/87.

Source: These regulations have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1998.

Table 2-4. FDA Regulations

FDA Regulations	
Regulatory action	Effect of regulation and other comments
21 CFR 175 – PART 175 – INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS. Promulgated: 42 FR 14534 03/15/77. U.S. Codes: 21 U.S.C. 321, 342, 348, 379e.	The subparts A through C deal with components of adhesives and of coatings that may migrate into food from packaging. Styrene oxide may be used only in coatings for containers having a capacity of 1,000 gallons or more when such containers are intended for repeated use in contact with alcoholic beverages containing up to 8% alcohol by volume.

Source: These regulations in this table have been updated through the 1998 Code of Federal Regulations 21 CFR, April 1, 1998.

3 Human Cancer Studies

No studies on the relationship of SO exposure to human cancer were available.

Because styrene is metabolized to SO in humans (see Section 6.1), studies of styrene exposure and human cancer will be briefly discussed.

IARC (1994b) reviewed studies of styrene exposure and human cancer that were based in the United States, Canada, or Europe. The studies generally focused on lymphohematopoietic cancers and found overall relative risks of 1.5 or less. Studies were partitioned by type of industry. Workers in the styrene-butadiene rubber industry had excess risk of leukemia and other lymphohematopoietic cancers (McMichael *et al.* 1976; Meinhardt *et al.* 1982; Matanoski *et al*. 1990, 1993). A case-control study nested within one of these cohorts (Matanoski *et al.* 1990) suggested that the excess was due to butadiene and not styrene exposure (Santos-Burgoa *et al*. 1992). Most studies of workers in styrene manufacture and polymerization plants found nonsignificant associations of styrene exposure with lymphohematopoietic cancers (Ott *et al*. 1980; Hodgeson and Jones 1985; Bond *et al*. 1992).

Exposures to styrene in the reinforced plastics industry were higher and less confounded by other exposures. Three early studies of this industry found little evidence for an association of styrene exposure with lymphohematopoietic cancers (Okun *et al*. 1985; Coggon *et al*. 1987; Wong *et al.* 1994). Kogevinas *et al*. (1994) studied a large cohort consisting of eight subcohorts in six European countries. There was no overall association of styrene exposure with lymphohematopoietic cancers, nor was there a dose-response for cumulative exposure or an association with job type. However, there were significant positive trends with increasing average exposure and with time since first exposure. Kolstad *et al*. (1994) studied Danish workers, some of whom were included in the large European cohort, and found a statistically nonsignificant overall association of lymphohematopoietic cancers with styrene exposure and a significant association in a subgroup of short-term workers with more than 10 years since first exposure. A case-control study of myeloid leukemia (Flodin *et al*. 1986) found an association with self-reported exposure to styrene. IARC (1994b) concluded there was *inadequate evidence* in humans for the carcinogenicity of styrene.

Delzell *et al*. (1996) found an overall excess risk of leukemia mortality among workers in eight North American styrene-butadiene rubber plants (SMR 1.31, 95% confidence interval [CI] 0.97-1.74, n=48); risk was greater in hourly workers, particularly those with 10+ years of work experience and 20+ years since first exposure, and among workers with job titles indicating they worked in polymerization processes, maintenance, or laboratories, with high exposure potentials. Retrospective, quantitative estimates of exposure suggested that the risk was related to butadiene and not styrene exposure (Macaluso *et al*. 1996). Further study of the cohort (Sathiakumar *et al*. 1998) confirmed the increased risk of leukemia (SMR 2.24, 95% CI 1.49-3.23) and found excess risk for non-Hodgkin's lymphoma (SMR 1.37, 95% CI 0.77-2.26), but not for other cancers of the lymphohematopoietic system.

A case-control study (Matanoski *et al*. 1997), nested in one of the styrene-butadiene rubber cohorts reviewed by IARC (Matanoski *et al*. 1990), examined the relationship of several lymphohematopoietic cancers to styrene and butadiene exposures. Leukemia and Hodgkin's disease were associated primarily with butadiene exposure, but lymphoma (odds ratio [OR] 2.62, 95% CI 0.40-17.15), lymphosarcoma (OR 3.88, 95% CI 1.57-9.59), and myeloma (OR 3.04, 95% CI 6.96) were associated with styrene exposure after adjusting for butadiene exposure.

Further analysis of the Danish reinforced plastics cohort, reviewed by IARC (Kolstad *et al*. 1994), found an increased incidence of pancreatic cancer (incidence rate ratio 2.2, 95% CI 1.1-4.5) (Kolstad *et al*. 1995). A case-control study, nested within the same cohort, found an increased risk of myeloid leukemia with clonal chromosome aberrations in styrene-exposed workers (RR 2.5, 95% CI 0.2-25.0), but the study was limited by the small number of exposed cases (n=11) (Kolstad *et al*. 1996).

In summary, IARC (1994b) concluded that there was *inadequate evidence* in humans for the carcinogenicity of styrene. Studies published since then have provided some additional evidence in humans that styrene is carcinogenic, but it remains difficult to disentangle exposures to styrene and butadiene in many of the cohorts studied. Further studies of the reinforced plastics industry may help resolve this issue.

4 Studies of Cancer in Experimental Animals

4.1 Carcinogenicity studies of orally administered SO in mice

Groups of 52 male and 52 female $B6C3F_1$ mice, seven weeks old, were administered SO at doses of 0, 375, or 750 mg/kg by gavage in corn oil (Lijinsky 1986). The test material was 96.6% pure, with unspecified amounts of benzaldehyde, benzene, and an unidentified chemical as impurities. SO was administered three times per week for 104 weeks; three to four weeks after the final dose, all surviving animals were sacrificed. There was a marked reduction in the survival of high-dose male and female mice, and the body weights were reduced in both groups (50% died by week 60), in the high-dose groups**.** SO administration was associated with increased incidences of proliferative changes in the forestomach of male and female mice. Tumor incidences are summarized in Table 4-1.

Table 4-1. Incidence of tumors in the forestomach and liver of $B6C3F_1$ mice administered SO by gavage for up to 104 weeks

Tumor type	Dose (mg/kg)		
	0	375	750
Males			
Forestomach			
Squamous cell papilloma	2/51	22/51**	8/52*
Squamous cell carcinoma	0/51	16/51**	15/52**
Papillomas/carcinomas combined	2/51	37/51**	21/52**
Liver			
Carcinomas and adenomas	12/51	28/52*	15/52
Females			
Forestomach			
Squamous cell papilloma	0/51	14/50**	17/51**
Squamous cell carcinoma	0/51	10/50**	3/51
Papillomas/carcinomas combined	0/51	24/50**	20/51**
Liver			
Carcinomas and adenomas	7/51	4/50	9/51

Source: Lijinsky (1986).
* $P < 0.05$, ** $P < 0.001$ (Fisher's exact test).

Both dose levels caused significantly increased incidences of squamous cell papillomas of the forestomach in males and females and squamous cell carcinomas in males and low-dose females. The incidences of papillomas and carcinomas combined were increased in both sexes at both dose levels. In males, tumors of the liver were significantly increased at the low dose; poor survival of the high-dose animals may have been responsible for the observation of no significant increase in liver tumors in the high-dose group.

4.2 Carcinogenicity studies of orally administered SO in rats

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, were administered SO at doses of 0, 50, or 250 mg/kg by gavage in olive oil (Maltoni *et al*. 1979; Conti *et al*. 1988, cited in IARC 1994a). SO was administered 4 to 5 days per week for 52 weeks; animals were then observed until death. The last animal died 156 weeks after the initial dose of SO. The SO dosage regimen had no effect on body weight gain or survival in either sex.

Administration of SO to male and female rats was associated with dose-related increased incidences of neoplasms of the forestomach (Table 4-2). No significant increases in the incidences of tumors at other sites were reported.

Table 4-2. Incidence of tumors in the forestomach of Sprague-Dawley rats administered SO by gavage for up to 52 weeks

Tumor type	Dose (mg/kg)		
	0	50	250
Males			
Forestomach squamous cell			
Papilloma/acanthoma	0/40	3/40	9/40**
Carcinoma	0/40	11/40**	30/40**
Females			
Forestomach squamous cell			
Papilloma/acanthoma	0/40	3/40	5/40*
Carcinoma	0/40	8/40**	33/40**

Source: Maltoni *et al*. (1979), Conti *et al*. (1988), both cited in IARC (1994a).
*$P < 0.05$, **$P < 0.01$ (Fisher's exact test)

The incidences of squamous cell papillomas/acanthomas and carcinomas of the forestomach were increased in dose-related manners in rats of both sexes.

Groups of 52 male and 52 female F344/N rats (nine weeks old) were administered SO at doses of 0, 275, or 550 mg/kg by gavage in corn oil (Lijinsky 1986, cited in IARC 1994a). The SO study material was 96.6% pure, with unspecified amounts of benzaldehyde, benzene, and an unidentified impurity. SO was administered three times per week for 104 weeks. All surviving animals were sacrificed three to four weeks after the final dose. Body weights and survival of the high-dose animals of both sexes were reduced. SO administration resulted in dose-related increased incidences of tumors of the forestomach in both sexes at both doses. No evidence of increased tumor incidences at other sites was reported. Incidences of forestomach neoplasms are summarized in Table 4-3. In a few (< 10%) animals, the carcinomas metastasized to the liver and other organs.

Table 4-3. Incidence of tumors in the forestomach of F344/N rats administered SO by gavage for up to 104 weeks

Tumor type	Dose (mg/kg)		
	0	275	550
Males			
Squamous cell papilloma	1/52	23/52*	18/51*
Squamous cell carcinoma	0/52	35/52*	43/51*
Papillomas/carcinomas combined	1/52	50/52*	50/51*
Females			
Squamous cell papilloma	0/52	21/52*	24/52*
Squamous cell carcinoma	0/52	32/52*	36/52*
Papillomas/carcinomas combined	0/52	46/52*	50/52*

Source: Lijinsky (1986), cited in IARC (1994a).
*$P < 0.01$ (Fisher's exact test).

4.2.1 Carcinogenesis study employing prenatal exposure and postnatal oral administration to rats

A group of 14 pregnant BDIV inbred rats (age not specified) received 200 mg/kg bw SO (97% pure) by gavage in olive oil on day 17 of gestation (Ponomarkov *et al*. 1984, cited in IARC 1994a). Beginning at four weeks of age, their offspring (43 males and 62 females) received SO once a week by gavage in olive oil at doses of 100 to 150 mg/kg for 96 weeks. Total dosages were estimated to be 2.5 g for females and 5.0 g for males. The concurrent control group included 49 male and 55 female rats with no prenatal or postnatal exposure to SO. The control group received olive oil by gavage. The study was terminated at 120 weeks.

When the first tumor appeared (time not specified), 42 male and 60 female SO-dosed progeny were alive. The incidences of forestomach tumors were increased in SO-dosed male and female rats (Table 4-4). Hyperplasia, dysplasia, and hyperkeratosis of the forestomach also were reported in treated rats. No evidence of increased tumor incidences at other sites was reported (Ponomarkov *et al*. 1984, cited in IARC 1994a).

Table 4-4. Summary of tumors in the forestomachs of BDIV rats pre- and post-natally exposed to SO[a]

Tumor type	Treatment group (offspring)			
	Males		Females	
	Control	100 to 150 mg/kg	Control	100 to 150 mg/kg
Papilloma	0/49	7/42**	2/55	2/60
Carcinoma *in situ*	0/49	4/42*	0/55	6/60*
Carcinoma	0/49	10/42**	1/55	16/60**

Source: Ponomarkov *et al.* (1984, cited in IARC 1994a).
[a] Pregnant rats received 200 mg/kg SO as an olive oil gavage on day 17 of gestation; then offspring, beginning at age four weeks, received 100 to 150 mg/kg weekly for 96 weeks.
*$P < 0.05$, **$P < 0.001$ (Fisher's exact test).

4.3 Carcinogenicity studies of SO applied to the skin of mice

A group of 40 C3H mice (13 weeks old) of unspecified sex received three weekly applications of a 5% solution of SO in acetone to the shaved dorsal skin for up to two years. Dose volumes were not specified. No skin tumors were observed in the 17 mice that survived for at least 24 months. Another group of 40 C3H mice received similar treatment with a 10% solution of SO in acetone. Survival clearly was affected; only 18 mice survived for as long as 12 months, and only two mice survived for 17 months. No skin tumors were observed (Weil *et al.* 1963, cited in IARC 1994a). The IARC Working Group noted the incomplete reporting of this study.

A group of 30 male Swiss ICR/Ha mice (eight weeks old) received three weekly dermal applications of 100 mg of a 10% solution of SO in benzene. Median survival time of dosed animals was 431 days. Three mice (10%) had skin tumors, one of which was a squamous cell carcinoma. Eleven of 150 controls (7%) developed skin tumors, one of which was a squamous cell carcinoma (Van Duuren *et al.* 1963, cited in IARC 1994a). The IARC Working Group noted the potential carcinogenicity of the vehicle.

4.4 Supporting evidence

Based on the results of these studies, IARC concluded that there was *sufficient evidence of carcinogenicity* of SO in experimental animals. In addition to the consistent induction of proliferative changes and benign and malignant tumors of the forestomach in mice and rats, IARC's conclusion regarding the carcinogenicity of SO and its classification as *probably carcinogenic in humans* (Group 2A) was based on the following information on SO:

- SO forms covalent adducts with DNA in humans, mice, and rats.
- SO induces gene mutations in bacterial and rodent cells *in vitro*.
- SO induces chromosomal aberrations, micronuclei, and sister chromatid exchanges in human cells *in vitro.*
- SO induces chromosomal aberrations and sister chromatid exchanges in mice *in vivo*.

4.5 Summary

Orally administered SO is carcinogenic to laboratory animals, causing increased incidences of squamous cell neoplasms in the forestomachs of male and female mice and of male and female rats. In the single study in mice, SO administration also was associated with an increased incidence of hepatocellular neoplasms in male mice.

Exposure to SO secondary to exposure to styrene *per se* requires metabolic conversion of the parent compound to the putative active metabolite. Human exposure to SO *per se* is most likely to occur in industrial settings and would entail multiple routes of exposure, including dermal, inhalation, and oral. There are no experimental carcinogenicity studies in which SO was administered by the inhalation route.

5 Genotoxicity

5.1 Prokaryotic Systems

5.1.1 Induction of mutation in Salmonella typhimurium

In several studies, SO was mutagenic in *Salmonella typhimurium* strain TA100 without metabolic activation at concentrations ranging from 0.6 µg/mL (Vainio *et al*. 1976, cited in IARC 1994a) to 12,000 µg/mL (Brams *et al*. 1987, cited in IARC 1994a). SO also was mutagenic in various studies without metabolic activation in strain TA1530 at a concentration of 768 µg/mL (de Meester *et al*. 1981, cited in IARC 1994a), strain TA104 at a concentration of 120 µg/mL (Einistö *et al*. 1993, cited in IARC 1994a), and strain TA1535 at concentrations ranging from 0.6 µg/mL (Vainio *et al*. 1976, cited in IARC 1994a) to 5000 µg/mL (Milvy and Garro 1976, cited in IARC 1994a). The mutagenic activity of SO was reduced by the presence of glutathione or S9 liver homogenate. The R enantiomer of SO was found to be more mutagenic in *S. typhimurium* strain TA100 than the S enantiomer (Seiler 1990, Sinsheimer *et al*. 1993, cited in IARC 1994a). In different studies, SO was not found to be mutagenic in *S. typhimurium* strains TA1537, TA98, or TA97 with metabolic activation when tested over a concentration range of 250 to 6,000 µg/mL (Watabe *et al*. 1978, de Meester *et al*. 1981, both cited in IARC 1994a).

5.1.2 Induction of mutation in Escherichia coli

SO was found to be mutagenic in *Escherichia coli* strain WP2 urA without metabolic activation at concentrations ranging from 480 µg/mL (Sugiura and Goto 1981, cited in IARC 1994a) to 720 µg/mL (Sugiura *et al*. 1978, cited in IARC 1994a).

5.1.3 Induction of mutation in Klebsiella pneumoniae

SO was found to be mutagenic in *Klebsiella pneumoniae* at a dose of 120 µg/mL (Voogd *et al*. 1981, cited in IARC 1994a).

5.2 Plants

5.2.1 Chromosomal aberrations

5.2.1.1 Chromosomal aberrations test

SO induced chromosomal aberrations in the meristematic root tip cells of *Allium cepa* at a concentration of 500 µg/mL without metabolic activation (Linnainmaa *et al*. 1978a,b, cited in IARC 1994a).

5.2.1.2 Micronucleus test

SO induced increased micronuclei formation in the meristematic root tip cells of *Allium cepa* at a concentration of 500 µg/mL without metabolic activation (Linnainmaa *et al*. 1978a,b, cited in IARC 1994a).

5.3 Eukaryotic Systems

5.3.1 Induction of mutation in Saccharomyces cerevisiae

SO caused mitotic gene conversions in *Saccharomyces cerevisiae* at a concentration of 1,200 μg/mL without metabolic activation (Loprieno *et al*. 1976, cited in IARC 1994a).

5.3.2 Induction of mutation in Schizosaccharomyces pombe

SO induced forward mutations in *Schizosaccharomyces pombe* at a concentration of 600 μg/mL (Loprieno *et al*. 1976, cited in IARC 1994a).

5.3.3 Mutagenicity in Drosophila melanogaster

5.3.3.1 Sex-linked recessive lethal assay

SO induced an increased frequency of sex-linked recessive lethal mutations in *Drosophila melanogaster* when administered as a vapor at a concentration of 200 ppm (980 mg/m^3), six hours per day for four days, or orally at a dose of 200 mg/kg in the feed for 24 hours without metabolic activation (Donner *et al*. 1979, cited in IARC 1985, 1994a).

5.4 Mammalian Systems

5.4.1 In vitro *assays*

5.4.1.1 Mouse lymphoma cell mutation test

SO induced a positive response in L5178Y (TK$^{+/-}$) cells in the mouse lymphoma assay at a concentration of 13.80 μg/mL without metabolic activation. Metabolic activation reduced the mutagenic activity of SO in this study (Amacher and Turner 1982, cited in IARC 1994a).

5.4.1.2 hprt locus forward mutation test

SO induced forward mutations at the *hprt* locus in Chinese hamster V79 cells at concentrations ranging from 100 μg/mL (Nishi *et al*. 1984, cited in IARC 1994a) to 1,020 μg/mL (Loprieno *et al*. 1976, cited in IARC 1994a) without metabolic activation. Perfusion of SO through isolated liver (of unknown species and strain) abolished its mutagenic effect on Chinese hamster V79 cells (Beije and Jenssen 1982, cited in IARC 1994a).

In human T lymphocytes treated with SO for 24 hours or 6 days at concentrations of 0.2 to 0.4 mM, the maximal dose-dependent mutation frequency at the *hprt* locus was10 to 20 mutants per 10^6 clonable cells. This is approximately fourfold higher than background in human T lymphocytes. No increase in *hprt* mutation frequency was seen at the lowest concentration tested (0.05 mM) (Bastlova *et al*. 1995). A subsequent, similarly conducted study found that SO induced mutations at the *hprt* locus at a frequency 3.6 to 4.8 times higher than background in human T lymphocytes (Bastlova and Podlutsky 1996).

5.4.1.3 Chromosomal aberrations tests

Chromosomal aberrations test

SO induced an increased frequency of chromosomal aberrations without metabolic activation in Chinese hamster V79 cells at a concentration of 90 μg/mL (Turchi *et al*. 1981, cited in IARC 1994a) and in human lymphocytes at concentrations ranging from 3.00 μg/mL (Pohlova and Sram 1985, cited in IARC 1994a) to 80.00 μg/mL (Linnainmaa *et al*. 1978a,b, cited in IARC 1994a).

Micronucleus test

SO induced increased micronucleus formation in cultured human cells at a concentration of 80 μg/mL without metabolic activation (Linnainmaa *et al*. 1978a,b, cited in IARC 1994a).

5.4.1.4 Sister chromatid exchanges

SO induced an increased frequency of sister chromatid exchanges (SCE) without metabolic activation in Chinese hamster ovary (CHO) cells at a concentration of 50.00 μg/mL (de Raat 1978, cited in IARC 1994a) and in cultured human lymphocytes at concentrations ranging from 1.00 μg/mL (Pohlova and Sram 1985, cited in IARC 1994a) to 8.4 μg/mL (Linnainmaa *et al*. 1978a, b, cited in IARC 1994a).

Exposure of cultured human lymphocytes to a SO concentration of 100 μM for 22, 36, 48, or 72 hours resulted in a six-fold increase in the induction of SCE at 22 hours of exposure. However, there was a clear and significant inverse relationship between exposure time and SCE frequency ($r = -0.9337$, $P = 0.0018$). No relationship between the replication index and the frequency of SCE was seen ($r = -0.36$, $P > 0.05$), although cell viability was decreased 74% relative to the control (Chakrabarti *et al*. 1997).

A study was conducted with cultured lymphocytes from human donors to determine the influence of glutathione S-transferase M1 (GSTM1) genotype on SCE induction by SO. In cultured human lymphocytes treated with SO at concentrations of 50 or 150 μM for 48 hours, the frequency of SCE was significantly increases ($P < 0.001$). The GSTM1 genotype had no influence on SCE induction by SO (Uuskula *et al*. 1995). A subsequent, similarly conducted study to determine the influence of glutathione S-transferase T1 (GSTT1) genotype on SCE induction by SO also found increased frequency of SCE induction following treatment with SO. In lymphocytes from individuals lacking the GST1 gene, the mean numbers of SCE/cell were 1.7 and 1.4 times the control values at SO concentration of 50 μM (2.78 and 4.83) and 150 μM (13.74 and 18.98), respectively. In lymphocytes from individuals with the GST1 gene, the mean numbers SCE were 2.78 and 13.74 times the control values at concentrations of 50 μM and 150 μM, respectively (Ollikainen *et al*. 1998).

5.4.1.5 *DNA damage/repair tests*

DNA single-strand breaks

SO induced single-strand breaks in the DNA of cultured primary hepatocytes of rats treated with SO at a concentration of 36 µg/mL under alkaline conditions without metabolic activation (Sina *et al*. 1983, cited in IARC 1994a).

SO induced single-strand breaks in DNA in human embryonal cells incubated for 3 or 18 hours at SO concentrations of 10, 50, or 100 µM (in 0.25% dimethylsulfoxide). A significant correlation was found between formation of 7-alkylguanine DNA adducts with SO and single-strand breaks in DNA (r = 0.98, *P* = 0.011) (Vodicka *et al*. 1996).

SO induced DNA single-strand breaks in human lymphocytes and calf thymus cells in culture in a Comet assay. SO was tested at concentrations of 0.05 to 0.6 mM for periods ranging from 1 to 24 hours (in a series of six experiments) and at concentrations of 0.1 or 0.2 mM for 6 days (in a series of three experiments). Overall, SO treatment decreased the survival of clonable cells. SO formed O^6-guanine DNA adducts at a level of 1 to 4 adducts per 10^8 nucleotides at concentrations of 0.2 to 0.6 mM in 24 hours. SO-induced single-strand DNA breaks occurred at all concentrations tested; the breaks in DNA were repaired within 24 hours (Bastlova *et al*. 1995).

Unscheduled DNA synthesis

SO induced unscheduled DNA synthesis (UDS) in a human heteroploid cell line and in human amniotic cells at unspecified doses without metabolic activation (Loprieno *et al*. 1978; Audette *et al*. 1979, both cited in IARC 1985).

5.4.2 In vivo *assays*

5.4.2.1 Host-mediated assay

Gavage doses of 100 mg/kg of SO to male Swiss albino mice increased the frequencies of gene conversion in *Saccharomyces cerevisiae* and of forward mutations in *Schizosaccharomyces pombe* in a host-mediated assay (Loprieno *et al*. 1976, cited in IARC 1994a).

5.4.2.2 Chromosomal aberrations

Chromosomal aberration test

Gavage treatment of CD-1 mice with 50, 500, or 1,000 mg/kg of SO resulted in increased incidences of chromosomal aberrations (CA) in bone marrow cells at all dose levels tested (Lopreino *et al*. 1978, cited in IARC 1985, 1994a). However, no increases in the incidence of CA were observed in the bone marrow cells of male Chinese hamsters exposed to SO vapors by inhalation at concentrations of 25, 50, 75, or 100 ppm (122, 245, 368, or 490 mg/m^3) for 2, 4, and 21 (25 ppm only) days (Norppa *et al*. 1979, cited in IARC 1985, 1994a).

Dominant lethal test (mouse)

SO did not induce dominant lethal mutations or translocations in meiotic germ cells of male BALB/c mice administered SO by intraperitoneal injection at a dose of 250 mg/kg (Fabry *et al*. 1978, cited in IARC 1985, 1994a).

Micronucleus test

SO administered by intraperitoneal injection at a dose of 250 mg/kg had no effect on the frequency of micronuclei in bone marrow cells of BALB/c mice and Chinese hamsters (Fabry *et al*. 1978, cited in IARC 1994a).

Mammalian germ cell cytogenetic test

SO administered to male BALB/c mice by intraperitoneal injection at a dose of 250 mg/kg did not induce reciprocal translocations in meiotic germ cells, had no effect on the frequency of CA in bone marrow cells, and did not induce reciprocal translocations in meiotic germ cells (Fabry *et al*. 1978, cited in IARC 1994a).

5.4.2.3 Sister chromatid exchanges

Inhalation exposure of mice to SO vapor at a concentration of 50 ppm (245 mg/m^3) induced a slight increase in SCE in regenerating liver cells and alveolar cells, but not in bone marrow cells (Conner *et al*. 1982, cited in IARC 1985, 1994a). However, no increases in the incidence of SCE were observed in the bone marrow cells of male Chinese hamsters exposed to SO vapor by inhalation at concentrations of 25, 50, 75, or 100 ppm (122, 245, 368, or 490 mg/m^3) for 2, 4, and 21 (25 ppm only) days (Norppa *et al*. 1979, cited in IARC 1985, 1994a).

5.4.2.4 DNA damage/repair

DNA single-strand breaks

SO caused single-strand DNA breaks in the liver, lung, kidney, testis, and brain of male mice administered SO by intraperitoneal injection at doses of 1.8 to 7.0 mM/kg (Walles and Orsen 1983, cited in IARC 1985).

Peripheral blood lymphocytes, liver cells, and kidney cells obtained from mice exposed to SO showed evidence of DNA damage (DNA single-strand breaks) upon analysis with the alkaline version of the single cell gel electrophoresis (Comet) assay. In the study, female C57BL/6 mice were given intraperitoneal injections of SO (in corn oil) at doses of 50, 100, 150, or 200 mg/kg four to six hours before sacrifice. Increased DNA damage, though not statistically significant ($P < 0.05$) in a one-tailed Kolmogorov-Smirnov two-sample test was observed in all cell types tested from the 50-mg/kg dose level. Statistically significant ($P < 0.001$) damage in DNA occurred in lymphocytes, liver, and kidney cells at doses ≥ 100 mg/kg. Statistically significant increases in the frequency of DNA damage in the bone marrow were seen only at the two highest doses tested (Vaghef and Hellman 1998).

Unscheduled DNA synthesis

Exposure of cultured human lymphocytes to SO at a concentration of 100 μM for 22, 36, 48, or 72 hours resulted in a sharp increase in DNA repair at early time points between 4 and 12 hours after exposure. At 12 hours after exposure to SO, UDS induction was 10-fold that of controls, decreasing rapidly from 24 hours to 72 hours. A significant time-dependent increase in S-phase DNA synthesis (DNA replication) was observed, with a peak response (33%) at 50 hours following SO exposure. Cell viability was decreased 74% relative to the control culture (Chakrabarti *et al*. 1997).

5.5 Summary

A summary of the genotoxicity of SO in prokaryotes, plants, eukaryotes, and mammalian systems (*in vitro* and *in vivo*) is presented in Table 5-1.

Table 5-1. Summary of genotoxicity studies for SO

Study type	Result		Reference
	With S9 activation	Without S9 activation	
Prokaryotes			
S. typhimurium TA97, mutation		+	IARC 1994a
S. typhimurium TA98, mutation		+	IARC 1994a
S. typhimurium TA100, mutation	+	+	IARC 1994a
S. typhimurium TA1530, mutation		+	IARC 1994a
S. typhimurium TA1535, mutation		+	IARC 1994a
S. typhimurium TA1537, mutation		+	IARC 1994a
E. coli WP2 urA, mutation	+		IARC 1994a
K. pneumoniae, mutation		+	IARC 1994a
Plants			
Alium cepa, chromosomal aberrations		+	IARC 1994a
Alium cepa, micronuclues test		+	IARC 1994a
Eukaryotes			
S. cerevisiae, gene conversion		+	IARC 1994a
S. pombe, forward mutation		+	IARC 1994a
D. melanogaster, sex-linked recessive lethal mutation		+	IARC 1985, 1994a
Mammalian systems (in vitro)			
L5178Y ($TK^{+/-}$) cells, mutation		+	IARC 1994a
Chinese hamster V79 cells, mutation		+	IARC 1994a
Human T-cells, mutation		+	Bastlova *et al.* 1995, Bastlova and Podlutsky 1996
Chinese hamster V79 cells, chromosomal aberrations		+	IARC 1994a

Study type	Result		Reference
	With S9 activation	Without S9 activation	
Human T-cells, chromosomal aberrations		+	IARC 1994a
Human cells, micronucleus test		+	IARC 1994a
Chinese hamster ovary cells, sister chromatid exchanges		+	IARC 1994a
Human lymphocytes, sister chromatid exchanges		+	Chakrabarti *et al.* 1997, Uuskula *et al.* 1995, Ollikainen *et al.* 1998
Primary rat hepatocytes, DNA damage/repair		+	IARC 1994a
Human embryonal cells, DNA damage/repair		+	Vodicka *et al*. 1996
Human lymphocytes, DNA damage/repair		+	Bastlova *et al*. 1995
Human cells		+	IARC 1985
Mammalian systems (in vivo)			
Swiss albino mice/S. cerevisia, host-mediated assay – gene conversion		+	IARC 1994a
Swiss albino mice/S. pombe, host-mediated assay – gene conversion		+	IARC 1994a
CD-1 mice, bone marrow chromosomal aberrations		+	IARC 1994a
Chinese hamster, bone marrow chromosomal aberrations		–	IARC 1994a
BALB/c mice, dominant lethal mutation		–	IARC 1985, 1994a
BALB/c mice, reciprocal translocation		–	IARC 1994a
BALB/c mice, bone marrow micronuclei test		–	IARC 1994a
Chinese hamsters, bone marrow micronuclei test		–	IARC 1994a
Mice, liver tissue sister chromatid exchanges		±	IARC 1985, 1994a
Chinese hamsters, bone marrow sister chromatid exchanges		–	IARC 1985, 1994a
Mice, DNA damage/repair		+	IARC 1985, Vaghef and Hellman 1998
Human lymphocytes, unscheduled DNA synthesis		+	Chakrabarti *et al*. 1997

SO is a direct-acting mutagen and induces point mutations in *Salmonella typhimurium*. SO also is clearly mutagenic in *E. coli*, *S. cerevisiae*, *S. pombe*, and *K. pneumoniae* without metabolic activation. SO is mutagenic at the *hprt* locus of mammalian cells, showing a correlation with the detection and level of guanine O^6 in studies conducted with peripheral blood lymphocytes (described in Section 6). SO is clastogenic, inducing chromosomal aberrations, SCE, and DNA single-strand breaks in human lymphocytes *in vitro* and *in vivo*.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and elimination

6.1.1 Absorption and pharmacokinetics of SO

The absorption of SO has not been extensively studied. There is evidence of its absorption through the respiratory tract. Urine from workers exposed to SO vapors contained large amounts of mandelic acid and phenylglyoxylic acid, both metabolites of SO (Hulz *et al.* 1967, Ohtsuji and Ikeda 1970, cited in IARC 1976). SO also is absorbed slowly through the skin (Hine and Rowe 1963, cited in IARC 1976).

Rappaport *et al.* (1996*)* reported the presence of SO-specific biomarkers, [α and β isomers of N^2-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′-phosphate DNA adducts and cysteine albumin adducts] in the blood of plastics workers exposed to both styrene *per se* and SO. Marczynski *et al.* (1997) studied the high molecular weight DNA fragmentation in white blood cells following SO incubation with human blood. The results indicate that SO exposure in blood may induce high molecular weight DNA fragmentation due to oxidative stress. Although humans may metabolize styrene monomer to SO, correlations between inhalation exposure to SO and biomarker formation were much stronger than correlations between exposure to styrene and biomarker formation.

Absorption of SO after oral and intraperitoneal administration to experimental animals has been demonstrated (reviewed in IARC 1994a). After intraperitoneal administration, SO is rapidly absorbed and cleared from the blood of mice (Bidoli *et al.* 1980, cited in IARC 1994a). *A*fter a single intraperitoneal injection of 200 mg/kg of SO in corn oil, the peak SO plasma concentration (40 ± 7 μg/mL) was reached within 7 minutes, and the chemical was no longer detectable at 60 minutes. The area under the curve for the time course of blood concentration of SO was 329 min x μg/g.

Langvardt and Nolan (1991, cited in IARC 1994a) studied SO pharmacokinetic parameters in male Fischer 344 rats after oral administration of SO at doses of 275 or 550 mg/kg. They reported highly variable blood concentrations ranging from 0.27 to 8.84 μg/mL in the low-dose animals and 2.1 to 32.4 μg/mL in the high-dose animals. Areas under the curve for the time course of blood concentration of SO after the low and high doses were 47 and 286 min x μg/g, respectively.

Kessler *et al.* (1992, cited in IARC 1994a) confirmed highly variable absorption of SO after oral administration to Sprague-Dawley rats and $B6C3F_1$ mice. They also demonstrated poor bioavailability of SO after oral administration. In these experiments both species received oral or intraperitoneal doses of 200 mg/kg of SO. The areas under the curve after injection or oral administration were 18 and 0.76 h x μg/mL in rats and 12 and 0.01 h x μg/mL in mice, respectively. Reduced bioavailability of SO after oral administration was considered to reflect its hydrolysis in the acidic environment of the stomach. Acid-catalyzed hydrolysis of SO was previously demonstrated *in vitro* (Ross *et al*. 1982, cited in IARC 1994a).

6.1.2 Metabolism and elimination

6.1.2.1 Metabolism

Rats and guinea pigs can metabolize SO, as demonstrated by excretion of the SO metabolite 3,4-dihydroxy-3,4-dihydro-1-vinylbenzene after SO administration (Nakatsu *et al.* 1983, cited in IARC 1985, 1994a). Microsomal and cytosolic enzymes mediate the mammalian metabolism of SO. The proposed metabolic scheme, based on isolated mammalian urinary metabolites, is shown in Figure 6-1 (Vainio *et al.* 1984, cited in IARC 1985).

SO is primarily detoxified by metabolism to styrene glycol (phenylethylene glycol) (Carlson 1998). Conversion of SO to styrene glycol is catalyzed by both microsomal and cytosolic epoxide hydrolases and enzyme preparations from mammalian liver, kidney, intestine, lungs, and skin *in vitro* (Oesch 1973, cited in IARC 1985). Hepatic and pulmonary microsomal preparations from non-Swiss albino and CD-1 mice were compared for their abilities to metabolize racemic, S-, and R-SO to styrene glycol (Carlson 1998). The enzymatic activity was found to be higher in liver than in lung tissues. When human cytosolic and microsomal systems were compared with respect to SO metabolism, microsomal activity was greater than that residing in the cytosol (Schladt *et al.* 1988, cited in IARC 1994a).

Human microsomal epoxide hydrolase (hmEH) was shown to protect Chinese hamster cells from SO-induced DNA damage. Herrero *et al.* (1997) inserted the cDNA of hmEH into V79 Chinese hamster cells, then challenged the cells with SO. Cells not containing hmEH responded to SO with DNA single-strand breaks and the generation of alkali-labile sites. One of the cell clones, designated 92hmEH-V79, was refractory to SO-induced DNA damage relative to mock-transfected cells. In extensions of the experiment, the protection against SO-induced genotoxicity afforded by the presence of hmEH was reversed by addition of valpromide, a selective inhibitor of microsomal epoxide hydrolase, to the incubation medium. Further, the observed protection against genotoxicity was specific for SO, as ethylene oxide-induced DNA damage was not affected by the presence of 92hmEH-V79.

The metabolic product of the action of microsomal epoxide hydrolase on SO has been shown to be styrene glycol. This metabolic step is completed in the absence of NADPH-generating system (Leibman and Ortiz 1970, cited in IARC 1976). Microsomal enzymes that carry out the initial metabolic step are inducible, as evidenced by the fact that *in vitro* metabolism of SO by hepatic enzymes from rats is enhanced by pretreatment of animals with phenobarbital or 3-methylcholanthrene. Enzyme activities engaged in the subsequent metabolism of styrene glycol to mandelic acid are not enhanced by the induction of microsomal enzymes (Oesch *et al.* 1971, cited in IARC 1976, 1985).

Early corroborative evidence for the styrene glycol metabolic path for SO came from by demonstration that administration of either SO or styrene glycol to rats resulted in the urinary excretion of phenylglyoxylic acid, mandelic acid, and hippuric acid. Injection of mandelic acid resulted in urinary excretion of phenylglyoxylic acid and hippuric acid. However, administration of phenylglyoxylic acid resulted in the appearance of only

unchanged phenylglyoxylic acid in the urine (Ohtsuji and Ikeda 1971, cited in IARC 1976). Isolated, perfused rat liver rapidly metabolizes SO to styrene glycol and mandelic acid (Ryan and Bend 1977, Steele *et al.* 1981, cited in IARC 1994a).

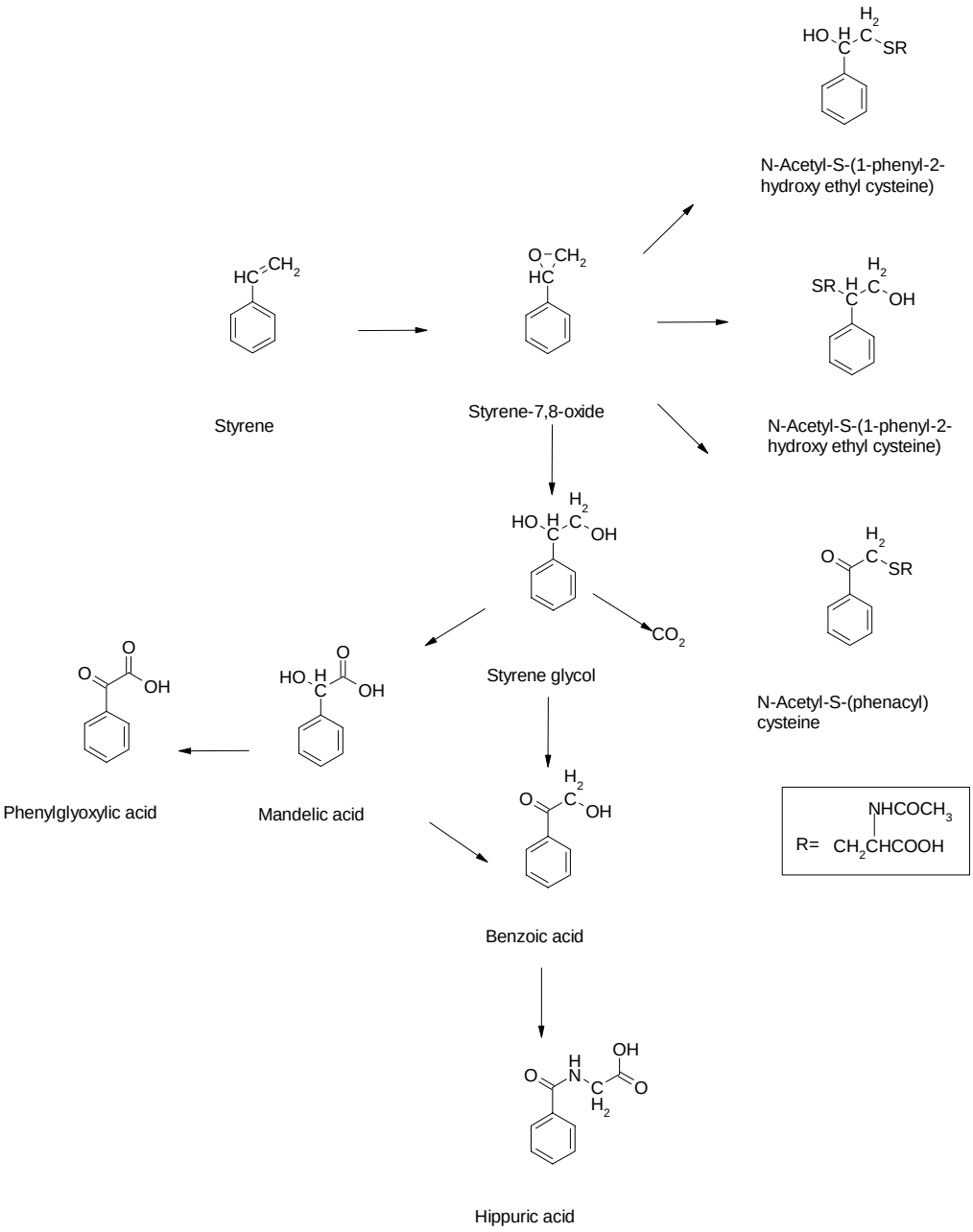

Figure 6-1. Metabolic pathways of SO

In addition to the oxidative metabolites, rats convert SO to glutathione conjugates, which are excreted via the kidney. Elimination of glutathione conjugates of SO appears to be a more prominent pathway in rats than in humans; however small quantities of mercapturic acid derivatives of SO have been detected in urine from workers at a plastics factory (Maestri *et al.* 1997).

Conjugation of SO with glutathione is catalyzed by glutathione S-epoxide transferase (GST) (James and White 1967, cited in IARC 1976). Among mammalian species, GST exists in multiple forms, which are classified under four multigene families: alpha, mu, pi, and theta. (The GST families are alternatively designated as GSTA, GSTM, GSTP, and GSTT.) The families have different but sometimes overlapping substrate specificities (Gopalan-Kriczky *et al.* 1994). Human liver cytosolic GST activity has been shown to occur in two forms, μ and α, with the μ form being more active in SO metabolism (Pacifici *et al.* 1987, cited in IARC 1994a).

Maestri *et al.* (1997) reported urinary excretion of small amounts of *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-cysteine and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-cysteine by humans occupationally exposed to styrene. The conversion of SO to mercapturic acids by humans is generally considered to be a minor metabolic pathway. Despite its purportedly minor role in hepatic metabolism of SO in humans, it is noteworthy that GST may play an important role(s) in preventing DNA adduct formation by reactive metabolites, including styrene epoxide, by catalyzing glutathione conjugate formation. Genes encoding GSTM and GSTT are polymorphic in humans, and the polymorphisms result in deletion of the genes in some individuals. In fact, about 50% of the Caucasian population is deficient in GSTM, while the GSTT gene is absent in 10% to 20% of the population (Seidegard *et al.* 1988; Hirvonen *et al.* 1993; Pemble *et al.* 1994; and Nelson *et al.* 1995).

Ollikainen *et al.* (1998) reported that GSTT affords some level of protection against induction of SCE in cultured human lymphocytes exposed to SO. Lymphocytes from humans genetically deficient in GSTT and lymphocytes from GSTT positive humans were cultured in the presence of SO at a concentration of 50 μM or 150 μM. Although the presence of SO caused SCEs in all lymphocytes, significantly more appeared in GSTT-deficient lymphocytes than in GSTT-positive lymphocytes (by factors of 1.7 and 1.4at SO concentrations of 50 μM and 150 μM, respectively).Similar experiments with lymphocytes from GSTM-deficient donors (Uuskula *et al.* 1995) demonstrated that the presence or absence of GSTM had no effect on the induction of SCEs by SO. However, the presence of GSTM did protect lymphocytes against induction of SCEs by 1,2-epoxide-3-butene. Observations that GSTs may protect against epoxide-induced adduct formation deserve serious follow-up in pursuit of information about the mechanism of SO carcinogenesis. The enzymes that metabolize SO are stereoselective, with the S enantiomer favored over the R enantiomer in hydrolysis by epoxide hydrolase (Watabe *et al.* 1981, cited in IARC 1994a). In contrast, glutathione S-transferases favor the R isomer (Hiratsuka *et al.* 1989, cited in IARC 1994a).

Human liver has been compared with liver from Fischer 344 and Sprague-Dawley rats and $B6C3F_1$ mice with respect to activities of cytochrome P-450 monooxygenase, microsomal and cytosolic forms of epoxide hydrolase, and glutathione S-transferase in the *in vitro* metabolism of SO (Mendrala *et al.* 1993, cited in IARC 1994a). The affinities of the monooxygenases (inverse K_m values) were essentially similar across species: 0.09 mmol in humans and 0.05 mmol in mice. The V_{max} values were similar in rats and mice (9.3 to 13 nmol/mg protein per minute) but lower in the human liver samples (2.1 nmol/mg protein per minute). The K_m values for epoxide hydrolase were low in humans

(0.01 mmol), intermediate in rats (0.13 to 0.23 mmol), and highest in mice (0.74 mmol) but the V_{max} values for epoxide hydrolase were similar among all species. Humans had the lowest glutathione S-transferase activity towards SO. These findings are consistent with the indirect observations of SO metabolism (identification of urinary metabolites) in these species.

6.1.2.2 Elimination

The primary route of excretion for SO metabolites in mammalian species is via urine. In rabbits, about 80% of a single oral dose was excreted in the urine (James and White 1967, cited in IARC 1994a). Excretion of acidic metabolites derived from glutathione conjugates varies qualitatively among species. In rats, the only glutathione conjugation products detected in urine are the mercapturic acids, whereas in guinea pigs, the major metabolites are mercaptoacetic acids together with mercaptolactic, mercaptopyruvic, and mercapturic acids.

6.2 Adduct formation

As a reactive epoxide, SO is electrophilic and binds to DNA or DNA constituents to form SO-DNA adducts. SO reacts with both the C7 (α)- and C8 (β)-carbon with DNA or DNA constituents. The C7 position of SO is chiral, and approximately equal amounts of the R and S enantiomers of SO are formed *in vitro* (Horvath *et al*. 1994; Schrader and Linscheid 1997). The principal reactions of SO with DNA or DNA constituents are at the endocyclic N^7-position, followed by the exocyclic N^2- and O^6- positions, usually dependent on guanine or guanine derivatives (Horvath *et al.* 1994, Latham *et al*. 1993, 1995; Schrader and Linscheid 1997).

Six adducts of SO with DNA constituents have been detected in cultured mammalian cells via the ^{32}P-postlabeling technique. Two of these six adducts have been identified as isomers of O^6-modified deoxyguanosine, O^6-(2-hydroxy-2-phenylethyl)2′-deoxyguanosine-3′,5′-bisphosphate and O^6-(2-hydroxy-1-phenylethyl)2′-deoxyguanosine-3′,5′-bisphosphate (Pongracz 1989, cited in IARC 1994a). A study of the lability of the deoxyguanosine-3′-monophosphate 7-alkylation products for postlabeling revealed that the 7-guanine adducts were considerably labile (Hemminki *et al*. 1990, cited in IARC 1994a). Through the ^{32}P-postlabeling method, six SO adducts also were detected in calf thymus DNA with N^2-guanosine derivatives being the major products (Figure 6-1) (Pongracz *et al*. 1992, cited in IARC 1994a). These modifications are not likely to occur *in vivo* (Phillips and Farmer 1994, cited in IARC 1994a).

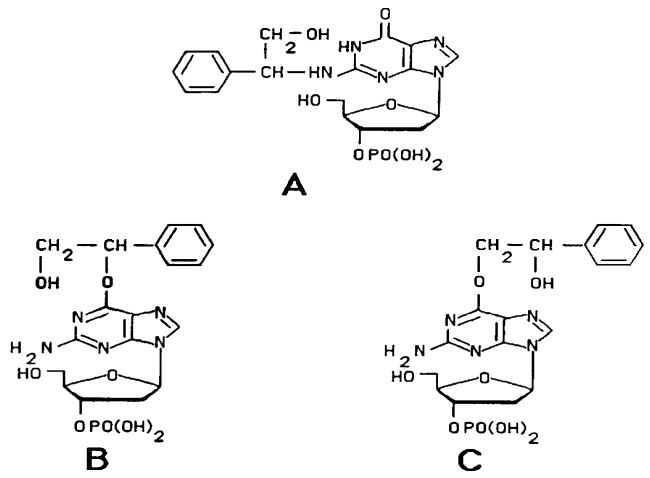

Figure 6-2. Structures of SO-DNA adducts detected by ^{32}P-postlabeling:

(A) *N*^{2}-(2-hydroxy-1-phenylethyl)-deoxyguanosine-3′-phosphate, (B) *O*^{6}-(2-hydroxy-1-phenylethyl)-deoxyguanosine-3′-phosphate, (C) *O*^{6}-(2-hydroxy-2-phenylethyl)-deoxyguanosine-3′phosphate.

Adducts involving modifications at the *N*-1 and *N*^{6} positions of adenine, the *N*^{4}, *N*-3, and *O*^{2} positions of cytosine, and the *N*-3 position of thymine also have been identified (Byfält-Nordqvist *et al*. 1985, cited in IARC 1994a; Phillips and Farmer 1994; Schrader and Linscheid 1997). The relative yields of other alkylation products in aqueous buffer were deoxyguanosine > deoxycytidine > deoxyadenosine > thymidine (Savela *et al*. 1986, cited in IARC 1994a). Depurination of 7-alkyldeoxyguanosine derivatives of SO and 7-methyldeoxyguanosine occurred at the same rate, while depurination was 15 and 55 times slower for 7-alkylguanine in ssDNA and dsDNA, respectively (Vodicka and Hemminki 1988, cited in IARC 1994a).

The efficiency of translesion synthesis or replication past these SO adducts has been shown, *in vitro*, to be both polymerase-specific and chirality- (R- or S-stereoisomers) dependent (Latham *et al*. 1993, 1995; Schrader and Linscheid 1997).

To test for the DNA-binding potential of SO in rodents, 24 B6C3F$_1$/CrlBR mice and six CD rats of both sexes were exposed to [7-^{3}H]styrene by inhalation in a closed chamber at concentrations of 20.1 to 38.6 mg/kg (rats) and 77.6 to 109.9 mg/kg (mice) for 4.5 to 6 hours (rats) and 6 to 9 hours (mice). Using data from this study and units of covalent binding index (CBI) to evaluate the DNA-binding activity of styrene, the investigators concluded that styrene had a very low DNA-binding potency (with a CBI of approximately 0.1) *in vivo* after inhalation exposure in rodents. Because almost 100% of styrene is metabolized through SO, it was concluded that this metabolite was responsible for the measured DNA-binding. The detection limit for DNA adducts was CBI < 0.1 for this study (Cantoreggi and Lutz 1993).

Liver samples from female CD rats exposed to styrene by inhalation at 1,000 ppm, six hours per day, five days per week, for 104 weeks were analyzed via the ^{32}P-postlabeling assay. Seven α-isomers of 2′-deoxyguanosyl-*O*^{6}-SO adducts per 10^7 nucleotides were identified. The limit of detection for the assay was given as three adducts per 10^7 nucleotides (pH 4) (Otteneder *et al*. 1999).

A study was conducted to determine the levels of SO-DNA adducts in 47 workers exposed to styrene at ambient air concentrations of styrene ranging from 1 –to 235 mg/m^3 (0.2 to 47.8 ppm) with a mean of 65.6 mg/m^3 (13.3 ppm) (SE = 10.5) in a boat manufacturing facility. DNA adducts were increased in the mononuclear cells obtained from the exposed workers. In the study, mononuclear cells were purified from whole blood drawn from the workers at roughly three-month intervals and evaluated with the ^{32}P-postlabeling method. The mean DNA adduct level for the styrene-exposed workers was 3 x 10^{-7}. *N*^{2}-(2-Hydroxy-1-phenylethyl)2′-deoxyguanosine-3′-5′-bisphosphate was identified as DNA adduct 1 in the mononuclear cells via cochromatographic methods and a modified ^{32}P-postlabeling procedure. Adduct 1 level ranged from 0.6 to 102 x 10^{-8} (mean 15.8 x 10^{-8}). A second isolated adduct (adduct 2), with a level ranging from 0.1 to 70.9 x 10^{-8} (mean 14.2 x 10^{-8}), could not be identified. Six SO-DNA adducts, at a relative adduct level of 4.5 x 10^{-7}, were detected in unmodified calf thymus muscle DNA coincubated with SO, which was used as standard for the ^{32}P-postlabeling procedure. The principal SO adduct detected in the calf thymus DNA standard was *N*^{2}-(hydroxy-1-phenylethyl)2′-deoxyguanosine-3′-5′-bisphosphate. *O*^{6}-(2-Hydroxy-1-phenylethyl)2′-deoxyguanosine-3′-5′-bisphosphate, *O*^{6}-(2-hydroxy-1-phenylethyl)2′-deoxyguanosine-3′-5′-bisphosphate, and three DNA-SO adducts were also detected (Horvath *et al*. 1994).

In vitro, SO also binds to histidine in human hemoglobin (Kaur *et al.* 1989, cited in IARC 1994a) and predominantly to cysteine in human plasma proteins (Hemminki 1986, cited in IARC 1994a). SO binds to polyamino acids in the order polycysteine >> polyhistidine > polylysine > polyserine (Hemminki 1983, cited in IARC 1994a). Binding of SO to amino acids was observed following exposure of mice, rats, and humans to SO (Osterman-Golkar 1992, Rappaport *et al.* 1993, cited in IARC 1994a; Yeowell-O'Connell *et al.* 1997; Pauwels and Veulemans 1998).

6.3 SO-induced squamous cell proliferation in rodent forestomachs

Orally administered SO consistently caused squamous-cell papillomas and carcinomas in rodent forestomachs in every long-term experiment (Huff 1984; McConnell and Swenberg 1993, 1994). In the case of SO, neoplasms at sites distant from the forestomach were observed in males of one species of mice, in the only experiment in mice. In that experiment, the low-dose males (but not the high-dose males, which exhibited poor survival) had an increased incidence of hepatocellular neoplasms. Further, the results of several *in vitro* and *in vivo* assays of genotoxicity have revealed SO's genotoxic activity (reviewed in Section 5).

The effects of SO on cell proliferation kinetics in the forestomach of mice and rats have been examined to determine whether SO-induced neoplasms could be empirically associated with this biologic activity. Gavage doses of SO in corn oil (0, 137, 275, or 550

mg/kg) (1 mL of solution/kg body weight) were administered to male Fischer 344 rats three times per week for four weeks. Additional rats received diets containing 0%, 0.5%, 1%, or 2% butylated hydroxyanisole (BHA) as a positive control treatment. (BHA is a widely used food additive [antioxidant] and is a nongenotoxic chemical that causes squamous cell neoplasms in rodent forestomachs.) Administration of SO resulted in a low level of covalent binding to forestomach DNA. Microscopic examination of forestomachs from animals given SO by gavage or BHA in the diet revealed that while SO caused slight thickening of the squamous cell layer of the forestomach, BHA caused marked epithelial hyperplasia and thickening. Cell counts revealed up to a 19-fold increase in cell density in the BHA-dosed animals. Both SO and BHA increased bromodeoxyuridine (BrdU) labeling indices of forestomach epithelial cells (expressed as percent of BrdU-positive cells generated during a 24-hour period immediately following the final SO or BHA doses) in all treated animals. In the prefundic region of the forestomach, the labeling index increased significantly, from 42% (solvent controls) to 54% with SO and from 41% to 55% with BHA (Lutz *et al.* 1993). Based on these observations, it was proposed that the carcinogenicity of SO to the forestomach probably involves a mechanism in which genotoxicity is combined with promotion by increased cell proliferation (Lutz *et al.* 1993). In a subsequent, similarly designed study by Dalbey *et al.* (1996) using [^{3}H]thymidine, the mean labeling index was dose-related, with increases at doses of up to 250 mg/kg. Higher doses did not cause any further increase in labeling index. According to these authors, the degree of involvement of cell proliferation in the tumorigenicity of SO remains uncertain.

6.4 Summary

Hepatic pathways of SO metabolism vary among mammalian species. In humans, the major pathway for this reactive epoxide is mediated by the microsomal enzyme epoxide hydrolase. Glutathione S-transferase mediated conjugation of SO appears to play only a minor role in humans but a greater role in rodents. SO is an alkylator of protein and DNA. Reaction with guanine in DNA has been shown to occur at the 7(*N*)-, N^2-, and O^6-positions and the 1- or 2- position of the 2-carbon side chain of SO, resulting in the formation N^2-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′-phosphate DNA adducts (α and β isomers). The C7 position of SO is chiral, and approximately equal amounts of the R and S enantiomers of SO are formed *in vitro*. Orally administered SO causes preneoplastic and neoplastic lesions of epithelial cells of the rodent forestomach, but the mechanism(s) for these responses remain largely unknown. A genotoxic mechanism of action is plausible.

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Appendix A: IARC. 1976. *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics. Styrene oxide.* IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Lyon, France. World Health Organization. Vol 11, (A-1 – A-10)

STYRENE OXIDE*

1. Chemical and Physical Data

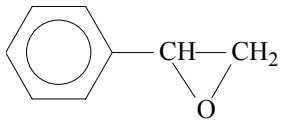
1.1 Synonyms and trade names

Chem. Abstr. Reg. Serial No.: 96-09-3

Chem. Abstr. Name: Phenyloxirane

Epoxyethylbenzene; (epoxyethyl)benzene; 1,2-epoxyethylbenzene; epoxystyrene; α,β-epoxystyrene; phenethylene oxide; phenylethylene oxide; 1-phenyl-1,2-epoxyethane; 2-phenyloxirane; phenyl oxirane; styrene epoxide; styreneoxide; styryl oxide

1 2 Chemical formula and molecular weight



C_8H_8O Mol. wt: 120.2

1.3 Chemical and physical properties

(a) *Description*: Colourless liquid

(b) *Boiling-point*: 194.1-195ºC

(c) *Freezing-point*: -36.8°C

(d) *Density*: d_{20}^{20} 1.0540

(e) *Refractive index*: n_D^{25} 1.5328

(f) *Spectroscopy data*: λ_{max}250 nm; E_1^1 = 13.2

(g) *Solubility*: Miscible with water (0.28 w/w at 25°C); miscible with most organic solvents

* Considered by the Working Group, February 1976

(h) *Volatility*: Vapour pressure is 0.3 mm at 20°C.

(l) *Reactivity*: Polymerizes and reacts with active hydrogen compounds (e.g., alcohols, amines). Reacts with 4-(4'-nitrobenzyl)pyridine (Preussmann *et al*., 1969)

1.4 Technical products and impurities

Styrene oxide (98 mole % pure) is available in the US with the following specifications: density, 1.0490-1.0515 (25/25°C); distillation range at 760 mm: fraction between 5 and 95% by volume shall boil within a 3.0°C range, which includes the temperature 194.1°C; water, 0.25% by wt (Mark *et al*., 1967).

2. Production, Use, Occurrence and Analysis

For important background information on this section, see preamble.

2.1 Production and use

Styrene oxide was prepared in 1905 from α-phenyl-β-iodoethanol by treatment with potassium hydroxide (Fourneau & Tiffeneau, 1905). It is reported to be produced commercially either by the chlorohydrin route or by epoxidation of styrene with peroxyacetic acid (Lapkin, 1967).

Styrene oxide is produced in the US by one company at two locations. In Japan, one company has been producing this compound commercially since 1964. Annual production has approached 100 thousand kg in the past, but smaller quantities are now being produced.

Styrene oxide is used as a reactive diluent in epoxy resins (Lee & Neville, 1967). It is also reported to be useful as an intermediate in the preparation of agricultural and biological chemicals, cosmetics, surface coatings and in the treatment of textiles and fibres.

In Japan, styrene oxide is used primarily as an intermediate for the production of phenylethyl alcohol and as a diluent in epoxy resins.

2.2 Occurrence

No data were available to the Working Group.

2.3 Analysis

Styrene oxide can be determined volumetrically in epoxide-glycol mixtures (Swan, 1954). It has been analysed in biological media by gas chromatography with flame-ionization detection and by thin-layer chromatography of the picrate (Leibman & Ortiz, 1970). Indirect spectrophotometric determination of styrene oxide at the nanomole level has also been reported (Mishmash & Meloan, 1972).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Man

3.1 Carcinogenicity and related studies in animals

Skin application

Mouse: Forty 12-week old C3H mice were painted on the clipped dorsal skin with a 5% solution of styrene oxide in acetone thrice weekly for life. No skin tumours were observed in 33 animals that survived 17-24 months. Forty C3H mice were similarly painted with a 10% solution of styrene oxide in acetone; only 2 mice survived at 17 months, and no tumours were observed (Weil *et al*., 1963).

Of 30 8-week old male Swiss ICR/Ha mice given thrice weekly applications of 0.1 mL of a 10% solution of styrene oxide in benzene on the clipped dorsal skin for life, 3 developed skin tumours; one of these had a squamous-cell carcinoma. The median survival time was 431 days. Of 150 benzene-painted controls, 11 developed skin tumours, and one of these had a squamous-cell carcinoma (Van Duuren *et al*., 1963).

3.2 Other relevant biological data

(a) Experimental systems

The oral LD_{50} of styrene oxide in Wistar rats is 4290 mg/kg bw (Smyth *et al*., 1954); the i.p. LD_{50} is 460 mg/kg bw (Ohtsuji & Ikeda, 1971); and the 4-hour LC_{50} is 4900 mg/m^3 (1000 ppm) (Weil *et al*., 1963). The LD_{50} by skin application in male New Zealand rabbits is 1060 mg/kg bw (Smyth *et al*., 1954).

Styrene oxide causes corneal injury in rabbits even with dilutions as low as 1%. Intradermal injection sensitized 6 of 11 guinea-pigs (Weil *et al*., 1963).

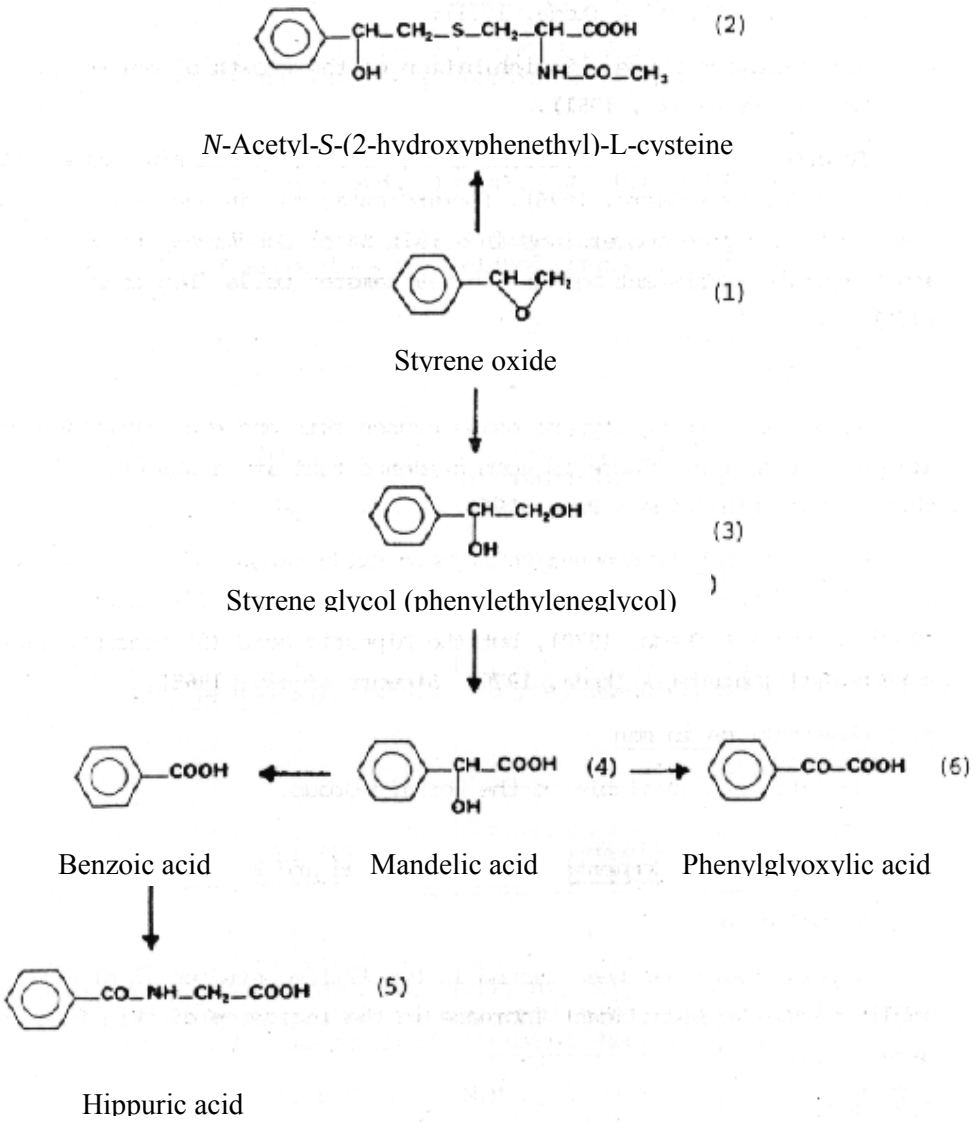
The main route of excretion of styrene oxide metabolites in animals is urinary; in rabbits, about 80% of a single oral dose was excreted via the kidneys (James & White, 1967).

Analysis of urine from rabbits given 1.7 mmol/kg bw styrene oxide (1) (see scheme) revealed 1.2% *N*-acetyl-*S*-(2-hydroxyphenethyl)-L-cysteine (hydroxyphenethyl mercapturic acid) (2), 30% (+)-mandelic acid (4), 25% hippuric acid (5) and 20% glucosiduronic acid; a greater proportion (6%) of *N*-acetyl-*S*-(2-hydroxyphenethyl)-L-cysteine (2) was excreted by rats given 2.1 mmol/kg bw. Phenylethylene glycol (3) *per se* was not identified amongst the urinary metabolites of styrene oxide (1) (James & White, 1967). This evidence is corroborated by the finding of Ohtsuji & Ikeda (1971) that in rats styrene oxide (1) and phenylethylene glycol (3) yielded phenylglyoxylic acid (6), mandelic acid (4) and hippuric acid (5). Injection of mandelic acid into rats resulted in the excretion of increased amounts of phenylglyoxylic acid (6), hippuric acid (5) and mandelic acid (4) in the urine; but administration of phenylglyoxylic acid (6) failed to produce any compound other than phenylglyoxylic acid (6) itself. It is improbable that mandelic acid (4) and phenylglyoxylic acid (6) are interconvertible *in vivo* (Ohtsuji & Ikeda, 1971).

The first step in the formation of *N*-acetyl-*S*-(2-hydroxyphenethyl)-L-cysteine (2) is catalysed by glutathione-*S*-epoxide transferase (James & White, 1967). Conjugation of the epoxide with glutathione was demonstrated *in vitro* in rat liver cytosol (Boyland & Williams, 1965) and in a purified enzyme preparation (Fjellstedt *et al*., 1973). Leibman & Ortiz (1970) demonstrated that styrene oxide is metabolized into phenylethylene glycol (3) by hepatic microsomal preparations in the absence of a NADPH-generating system.

Styrene oxide (1) is converted *in vitro* into styrene glycol (phenylethylene glycol) (3) by microsomal epoxide hydrase from the liver, kidneys, intestine, lungs and skin of several mammalian species (Oesch, 1973).

Scheme



Metabolic pathways for styrene oxide

The biotransformation of styrene oxide into phenylethylene glycol was stimulated by pretreatment of rats with phenobarbitone; however, the further metabolism of phenylethylene glycol to mandelic acid was not (Oesch *et al*., 1971; Ohtsuji & Ikeda, 1971).

Styrene oxide caused 18% inhibition of the growth of Walker carcinoma in rats (Hendry *et al*., 1951).

It produced reverse mutations in *Salmonella typhimurium* strains TA1535 and TA100 (Milvy & Garro, 1976), forward mutations in *Schizosaccharomyces pombe*, mitotic gene conversions in strain D4 of *Saccharomyces cerevisiae* and azaguanine-resistant mutants in V79 hamster cells (Loprieno *et al*., 1976).

(b) Man

Acute exposure to styrene oxide causes skin and eye irritation and skin sensitization. There is some evidence that it is absorbed slowly through the skin (Hine & Rowe, 1963).

Urine of workers exposed to styrene oxide vapour contained large amounts of mandelic acid (4) and phenylglyoxylic acid (6) (Huzl *et al*., 1967; Ohtsuji & Ikeda, 1970), but the hippuric acid (5) concentrations were normal (Ohtsuji & Ikeda, 1970; Stewart *et al*., 1968).

3.3 Observations in man

No data were available to the Working Group.

4. Comments on Data Reported and Evaluation

4.1 Animal data

Styrene oxide has been tested in two limited studies in mice by skin application. No significant increase in the incidence of skin tumours was observed.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

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Appendix B: IARC. (1979). *Some Monomers, Plastics, and Synthetic Elastomers, and Acrolein. Styrene oxide.* IARC Monographs on the Evaluations of the Carcinogenic Risk of Chemicals to Humans. Lyon, France. World Health Organization. Vol 19, B-1 – B-12.

STYRENE OXIDE

This substance was considered by a previous IARC Working Group, in February 1976 (IARC, 1976). Since that time new data have become available, and these have been incorporated into the monograph and taken into account in the present evaluation.

1. Chemical and Physical Data

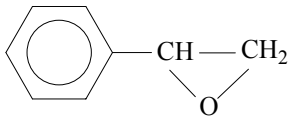
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 96-09-3

Chem. Abstr. Name: Phenyloxirane

(Epoxyethyl)benzene; 1,2-epoxyethylbenzene; epoxystyrene; α,β-epoxystyrene; phenethylene oxide; 1-phenyl-1,2-epoxyethane; phenylethylene oxide; 2-phenyloxirane; styrene epoxide; styryl oxide

1.2 Structural and molecular formulae and molecular weight



C_8H_8O Mol. wt: 120.2

1.3 Chemical and physical properties of the pure substance

From Weast (1976), unless otherwise specified

(a) Description: Colourless-to-pale-straw-coloured liquid (Hawley, 1971)

(b) Boiling-point: 194.1°C

(c) Melting-point: –36.6°C (Hawley, 1971)

(d) Density: d_4^{16} 1.0523

(e) Refractive index: n_D^{20} 1.5342

(f) Spectroscopy data: λ_{max} 250 nm (shoulder), 254 nm, 260 nm, 265 nm (shoulder) (E_1^1 = 13.2, 14.5, 16, 10); infrared, Raman, nuclear magnetic resonance and mass spectral data have been tabulated (Grasselli & Ritchey, 1975).

(g) Solubility: Slightly soluble in water (0.28% at 25°C); miscible with methanol, ether, carbon tetrachloride, benzene and acetone (Hine & Rowe, 1963)

(h) Volatility: Vapour pressure is 0.3 mm at 20°C (Hine & Rowe, 1963).

(i) Stability: Flash-point is 80°C (Hine & Rowe, 1963).

(j) Reactivity: Polymerizes exothermally and reacts vigorously with compounds possessing a labile hydrogen (e.g., alcohols), in the presence of catalysts such as acids, bases and certain salts (Hine & Rowe, 1967)

(k) Conversion factor: 1 ppm in air = 4.9 mg/m^3

1.4 Technical products and impurities

Styrene oxide (98 mol % pure) available in the USA has the following specifications: density, 1.0490–1.0515 (25/25°C); distillation range at 760 mm: fraction between 5 and 95% by volume shall boil within a 3.0°C range, which includes the temperature 194.1°C; water, 0.25% by wt max (Mack *et al*., 1967).

In Japan, styrene oxide is available commercially with a minimum purity of 98% and contains mono- and dichloroethylbenzene and unreacted styrene monomer as impurities. Additional specifications include: specific gravity, 1.0530–1.0560 (20/20°C); refractive index, 1.5330–1.5355 (20°C); boiling-point, 194.1°C; and water, 0.1% max.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) Production

Styrene oxide was prepared in 1905 from α-phenyl-β-iodoethanol by treatment with potassium hydroxide (Fourneau & Tiffeneau, 1905). It is produced commercially either by the chlorhydrin route or by epoxidation of styrene with peroxyacetic acid (Lapkin, 1967).

Commercial production of styrene oxide in the USA was first reported in 1974 (US International Trade Commission, 1976). Annual production of the one US producer is 450–900 thousand kg.

Styrene oxide has been produced commercially in Japan since 1964. One company produced 1.8 million kg in 1976, and 10 thousand kg were exported.

(b) Use

Styrene oxide is used as a reactive diluent in epoxy resins to reduce the viscosity of mixed systems prior to curing (Lee & Neville, 1967). It is also used as an intermediate in the preparation of agricultural and biological chemicals, cosmetics, surface coatings, and in the treatment of textiles and fibres.

In Japan, styrene oxide is used as a raw material for the production of phenylstearyl alcohol used in perfume.

In the USA, the Food and Drug Administration has ruled that styrene oxide may be used as a catalyst and cross-linking agent for epoxy resins in coatings for containers with a capacity of 1000 gallons (3785 L) or more when such containers are intended for repeated use in contact with beverages containing up to 8% of alcohol by volume (US Food & Drug Administration, 1977).

2.2 Occurrence

Styrene oxide has been detected as a volatile component of a Burley tobacco concentrate (Demole & Berthet, 1972), as a by-product in commercial samples of styrene chlorhydrin (Dolgopolov & Lishcheta, 1971), and in effluent water from latex manufacturing plants in Louisville, KY, and from chemical manufacturing plants in Louisville and in Memphis, TN (Shackelford & Keith, 1976).

2.3 Analysis

Styrene oxide can be determined volumetrically in epoxide-glycol mixtures (Swan, 1954). It has been analysed by thin-layer chromatography at the microgram level (Dolgopolov & Lishcheta, 1971; Kulicka *et al*., 1967) and in biological media by gas chromatography with flame-ionization detection and thin-layer chromatography of the picrate (Leibman & Ortiz, 1970). It has also been determined at the nanomole level by indirect spectrophotometry (Mishmash & Meloan, 1972).

The quantity of styrene oxide produced by the action of styrene oxidase on styrene has been determined by hydration to styrene glycol, followed by esterification using pentafluorobenzoyl chloride to a highly sensitive derivative analysed by gas chromatography-electron capture detection. The limit of detection was 0.01 ng/injection (van Bogaert *et al*., 1978).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

Skin application

Mouse: Forty 13-week-old C3H mice were painted on the clipped dorsal skin with a 5% solution of styrene oxide in acetone thrice weekly for life. No skin tumours were observed in 33 animals that survived 17–24 months (37 mice were alive at 12 months). Another group of C3H mice were similarly painted with a 10% solution of styrene oxide in acetone; 18 mice survived 12 months, only only mice survived to 17 months, and no skin tumours were observed (Weil *et al*., 1963).

Of 30 eight-week-old male Swiss ICR/Ha mice given thrice weekly applications of 0.1 mL of a 10% solution of styrene oxide in benzene on the clipped dorsal skin for life, three developed skin tumours; one of these was a squamous-cell carcinoma. The median survival time was 431 days. Of 150 benzene-painted controls, 11 developed skin tumours, and one of these was a squamous-cell carcinoma (Van Duuren *et al*., 1963).

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The oral LD_{50} of styrene oxide in rats is 4290 mg/kg bw (Smyth *et al*., 1954) or 3000 mg/kg bw (Weil *et al*., 1963); the intraperitoneal LD_{50} is 460–610 mg/kg bw (Ohtsuji & Ikeda, 1971). The LD_{50} by skin application in rabbits is 1060 mg/kg bw (Smyth *et al*, 1954) or 930 mg/kg bw (Weil *et al*., 1963). Inhalation of 4900 mg/m^3 (1000 ppm) in air killed 2/6 rats in 4 h (Weil *et al*., 1963).

Styrene oxide causes corneal injury in rabbits, even with dilutions as low as 1% (Hine & Rowe, 1963). Intradermal injections sensitized guinea-pigs (Weil *et al*., 1963).

One intraperitoneal dose of 375 mg/kg bw styrene oxide caused a significant decrease in the activities of mixed-function oxidases and in cytochrome P-450 content (Parkki *et al*., 1976). Styrene oxide decreases liver glutathione content *in vivo* (Marniemi *et al*., 1977).

[1] The Working Group was aware of studies in progress or planned to test the carcinogenicity of styrene oxide in mice and rats by oral administration (IARC, 1978).

Embryotoxicity and teratogenicity

When styrene oxide was injected into the air space of fertilized eggs at concentrations of 0.5-5 μmol/egg, it was embryotoxic and caused malformations in developing chicks. The LD_{50} for embryos was 1.5 μmol/egg when injected on the 4th day of incubation (Vainio *et al*., 1977).

Absorption, distribution and excretion

The main route of excretion of styrene oxide metabolites in animals is via the kidney; in rabbits, about 80% of a single oral dose was excreted in the urine (James & White, 1967).

Metabolism (see also the monograph on styrene, p. 240)

Styrene oxide is converted *in vitro* into styrene glycol (phenylethylene glycol) by microsomal epoxide hydrase from the liver, kidneys, intestine, lungs and skin of several mammalian species (Oesch, 1973).

The biotransformation of styrene oxide into styrene glycol was stimulated by pretreatment of rats with phenobarbital or 3-methylcholanthrene; however, the further metabolism of styrene glycol to mandelic acid was not stimulated (Oesch *et al*., 1971; Ohtsuji & Ikeda, 1971). Isolated perfused rat livers rapidly metabolized styrene oxide to approximately equal amounts of styrene glycol, mandelic acid and *S*-(l-phenyl-2-hydroxyethyl)glutathione (Ryan & Bend, 1977).

Styrene oxide injected intraperitoneally or incubated *in vitro* binds covalently to microsomes, protein and nucleic acid fractions of rat liver (Marniemi *et al*., 1977).

Mutagenicity and other short-term tests

Styrene oxide was mutagenic to *Salmonella typhimurium* TA1535 and TA100 in the absence of metabolic activation (de Meester *et al*., 1977; Milvy & Garro, 1976; Vainio *et al*., 1976).

A dose-dependent increase of forward mutations in *Schizosaccharomyces pombe* and gene conversion in *Saccharomyces cerevisiae* was obtained by treatment with styrene oxide (Loprieno *et al*., 1976). In a host-mediated assay, 100 mg/kg bw styrene oxide given by gavage to male Swiss albino mice increased the frequency of gene conversions in *S. cerevisiae* but not of forward mutations in *Sch. pombe* (Loprieno *et al*., 1976).

Styrene oxide caused a dose-dependent increase of mutation to 8-azaguanine resistance in cultured V79 Chinese hamster cells (Loprieno *et al*., 1976).

(b) Humans

Acute exposure to styrene oxide causes skin and eye irritation and skin sensitization. It is absorbed slowly through the skin (Hine & Rowe, 1967).

3.3 Case reports and epidemiological studies

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Styrene oxide was tested in mice by skin application. No increase in the incidence of skin tumours was observed.

Styrene oxide is mutagenic.

4.2 Human data

No case reports or epidemiological studies regarding the carcinogenicity of styrene oxide were available to the Working Group. The fact that styrene oxide is used primarily as a chemical intermediate suggests that occupationally exposed groups could be identified for epidemiological investigation.

4.3 Evaluation

The data available to the Working Group do not permit an assessment of the carcinogenicity of styrene oxide. Animal experiments and epidemiological studies should be undertaken (see also ‘General Remarks on the Substances Considered’, p. 35).

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STYRENE OXIDE

This substance was considered by previous working groups, in February 1976 (IARC, 1976). February 1978 (IARC, 1979a) and February 1982 (IARC, 1982a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into account in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

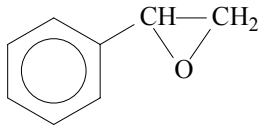
Chem. Abstr. Services Reg. No.: 96-09-3

Chem. Abstr. Name: Oxirane, phenyl-

IUPAC Systematic Name: (Epoxyethyl)benzene

Synonyms: Epoxyethylbenzene; 1,2-epoxyethylbenzene; (1,2-epoxyethyl)benzene; epoxystyrene; α,β-epoxystyrene; NCI-C54977; phenethylene oxide; 1-phenyl-1,2-epoxyethane; phenylethylene oxide; phenyl oxirane; phenyloxirane; 1-phenyl-oxirane; 2-phenyloxirane; styrene epoxide; styrene 7,8-oxide; styrene-7,8-oxide; styryl oxide

1.2 Structural and molecular formulae and molecular weight



C_8H_8O Mol. wt: 120.2

1.3 Chemical and physical properties of the pure substance

From National Research Council (1981), unless otherwise specified

(*a*) *Description*: Colourless to pale, straw-coloured liquid (Hawley, 1981)

(*b*) *Boiling-point*: 194.1°C

(*c*) *Freezing-point*: –36.8°C

(*d*) *Density*: Specific gravity (20/20°C). 1.0540

(*e*) *Refractive index*: n_D^{20} 1.5339

(*f*) *Spectroscopy data*: Ultraviolet, infrared, nuclear magnetic resonance and mass spectral data have been reported

(*g*) *Solubility*: Slightly soluble in water (0.3%); completely soluble in acetone, benzene, carbon tetrachloride, diethyl ether, heptane and methanol

(*h*) *Viscosity*: 1.99 cP at 20°C

(*i*) *Volatility*: Vapour pressure, 0.3 mm Hg at 20°C

(*j*) *Stability*: Flash-point, 80°C

(*k*) *Reactivity*: Reacts with compounds having labile hydrogen

(*l*) *Conversion factor*: 1 ppm = 4.91 mg/m^3 at 760 mm Hg and 25°C

1.4 Technical products and impurities

Typical properties of the styrene oxide available from the sole US producing company are: purity, 97.5%: apparent specific gravity (20/20°C), 1.0540; boiling-point, 194.0°C; vapour pressure, < 1 mm Hg at 20°C; freezing-point, –36.8°C; and solubility in water (20°C), 0.3% (Union Carbide Corporation. 1979).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Styrene oxide was first made in 1905 by the reaction of styrene iodohydrin with potassium hydroxide (Fourneau & Tiffeneau, 1905). It is believed to be produced commercially either by treating styrene chlorohydrin with alkali or by epoxidizing styrene (see IARC, 1979b) with peracetic acid (Lapkin, 1967).

Commercial production of styrene oxide in the USA was first reported in 1974 (US International Trade Commission, 1976). Currently, the sole producer is estimated to produce 450–900 thousand kg per year. Imports of styrene oxide through the principal US customs districts have been reported in only two years. In 1979, they amounted to 24 thousand kg (US International Trade Commission, 1980), and in 1982, they totalled 276 thousand kg (US International Trade Commission, 1983). Separate data on US exports of styrene oxide are not published.

Commercial production in Japan of styrene oxide started in 1964. Two Japanese companies currently manufacture styrene oxide, and their combined 1983 production is estimated to have been 3–4 million kg, about one million kg of which was exported.

(*b*) *Use*

Styrene oxide is used as a reactive plasticizer or diluent in epoxy resins to lengthen the polymer segments between cross-links and to produce a slight softening and flexibility with improved impact strength (Sears & Touchette, 1982).

Styrene oxide is also used as a chemical intermediate in one of the commercial processes for making β-phenethyl alcohol, a fragrance material. Ringk and Theimer (1978) reported that 20% of all β-phenethyl alcohol is produced by this process, and they estimated that total US production of β-phenethyl alcohol amounted to 1.2 million kg in 1976 (this would mean that 240 thousand kg were made from styrene oxide).

Styrene oxide has also been used to make a polymer with linoleic acid dimer, ethylene diamine and 2-ethoxyethyl acetate. It may also have been used as a chemical intermediate to make special polyols.

In Japan, approximately half of the styrene oxide is used to make β-phenethyl alcohol, and the remainder is used in epoxy resins and other applications.

Styrene oxide has been approved by the US Food and Drug Administration (1980) for use as a cross-linking agent for epoxy resins in coatings for containers with a volume of 1000 gallons (3785 l) or more intended for repeated use in contact with alcoholic beverages containing up to 8% of ethanol by volume.

2.2 Occurrence

(*a*) *Occupational exposure*

In its 1974 National Occupational Hazard Survey, the National Institute for Occupational Safety and Health (1981) reported that US workers in two industries were exposed to styrene oxide. The principal industry in which exposure was found was fabricated rubber products; some exposure was also noted in the paints and allied products industry.

Exposure to styrene oxide can occur when styrene is used in the reinforced plastics industry: styrene oxide was found in the air of a plant in Finland. The average breathing-zone concentration during lamination processes was 0.2 mg/m^3 during hand application (the simultaneous concentration of styrene was 560 mg/m^3) and 0.6 mg/m^3 during spray application (the simultaneous concentration of styrene was 550 mg/m^3) (Pfäffli *et al.,* 1979). Styrene oxide concentrations ranging from < 0.02–0.6 mg/m^3 were observed in the air during the manufacture of reinforced polyester plastics in Norway (the simultaneous concentrations of styrene ranged from 70–1200 mg/m^3) (Fjeldstad *et al.,* 1979).

(*b*) *Air*

Styrene oxide has been tentatively identified in air samples collected in the Los Angeles Basin, USA, using gas chromatography and mass spectrometry analysis (Pellizzari *et al.,* 1976). Sawicki (1976) has also reported the tentative identification of styrene oxide in US atmospheric air samples using similar analytical methods.

(*c*) *Water*

Styrene oxide has been detected in effluent-water from latex manufacturing plants in Louisville, KY, and from chemical manufacturing plants in Louisville and in Memphis, TN (Shackelford & Keith, 1976).

(*d*) *Tobacco and tobacco smoke*

Styrene oxide has been detected as a volatile component of a Burley tobacco concentrate (Demole & Berthet, 1972).

(*e*) *Biological fluids*

Low concentrations of styrene oxide (0.05 μmol/L) have been detected in the venous blood of four workers exposed to styrene of unspecified purity (Wigaeus *et al*., 1983).

(*f*) *Other*

Styrene oxide has been detected as an impurity in commercial samples of styrene chlorohydrin (Dolgopolov & Lishcheta, 1971).

2.3 Analysis

Reported methods for the analysis of styrene oxide in a variety of matrices are listed in Table 1.

Table 1. Methods for the analysis of styrene oxide

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Ambient air	Collect on sorbent; desorb thermally	GC/MS	2 ng/m^3	Pellizzari *et al*. (1976); Krost *et al*. (1982)
Workplace air	Collect on sorbent; extract (ethyl acetate)	GC/FID	0.2 ng in extract (0.1 μg/sample)	Stampfer & Hermes (1981)
	Collect on charcoal; extract (dichloromethane)	GC/FID; GC/MS	Not given	Pfaffli *et al*. (1979)
Drinking water	Concentrate; extract (ethanol); react with 4-nitrothiophenol	HPLC/UV	Not given	Cheh & Carlson (1981)
Biological media	Form picrate	GC/FID or TLC	Not given	Leibman & Ortiz (1970)
Mouse blood	Extract (dichloromethane); use para-methylanisole as an internal standard	GC/FID or GC/MS	10 ng/mL	Bidoli *et al*. (1980)
Rat-liver homogenate	React with nicotinamide; incubate	Fluorimetry	24–60 ng	Nelis & Sinsheimer (1981)
Commercial styrene chlorohydrin		TLC/Spectrophotometry	1.5 μg	Dolgopolov & Lishcheta (1971)
Aqueous solution	React with periodate; react with cadmium iodide-starch	Spectrophotometry	Not given	Mishmash & Meloan (1972)
	React with sodium sulphite	Titration	Not given	Swan (1954)
Acetone solution	React with 4-(*p*-nitrobenzyl)-pyridine/triethylamine	Spectrophotometry	12 μg max	Agarwal *et al*. (1979)

[a]Abbreviations: GC/MS, gas chromatography/mass spectrometry; GC/FID, gas chromatography/flame ionization detection; HPLC/UV, high-performance liquid chromatography/ultraviolet absorbance detection; TLC, thin-layer chromatography

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

(*a*) *Oral administration*

Rat: Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, received 50 or 250 mg/kg bw styrene oxide [purity unspecified] in olive oil by intragastric intubation once daily, on four to five days per week for 52 weeks. Control groups of 40 male and 40 female rats received olive oil alone. The preliminary results reported refer to an observation period of 135 weeks from the start of the bioassay and are concerned solely with oncological lesions of the stomach. Treatment with styrene oxide induced a dose-dependent increase in the incidence of squamous-cell carcinomas and papillomas of the forestomach. The first epithelial tumour of the forestomach appeared after 51 weeks. The incidences (referring to corrected numbers of animals alive at 51 weeks) of squamous-cell carcinomas (invasive plus *in situ*) were 0/37 in control, 6/31 in low-dose and 12/28 in high-dose males, respectively; and 0/28, 6/31 and 15/30, respectively, in females. The incidences of papillomas of the forestomach were 0/37, 0/31 and 3/28, respectively, in males; and 0/28, 2/31 and 6/30, respectively, in females (Maltoni *et al*., 1979).

(*b*) *Skin application*

Mouse: A group of 40 13-week-old C3H mice received skin applications by brush of a 5% solution of styrene oxide in acetone on the clipped dorsal skin thrice weekly for life. No skin tumour was observed at 24 months; 37 mice survived 12 months, 33 mice survived 17 months and 17 mice survived 24 months. Another group of C3H mice was similarly treated with a 10% solution of styrene oxide in acetone; 18 mice survived 12 months, only two mice survived to 17 months, and no skin tumour was observed (Weil *et al*., 1963).

Of 30 male Swiss ICR/Ha mice, eight weeks of age at the start of the treatment, given thrice-weekly applications of 100 mg of a 10% solution of styrene oxide in benzene on the clipped dorsal skin for life, three developed skin tumours; one of these was a squamous-cell carcinoma. The median survival time was 431 days. Of 150 benzene-painted controls, 11 developed skin tumours, one of which was a squamous-cell carcinoma. The study was considered to be negative by life-table analysis (Van Duuren *et al*., 1963).

(*c*) *Pre- and post-natal administration*

Rat: A group of 14 female BDIV inbred rats [age unspecified] were given 200 mg/kg bw styrene oxide (purity, 97%) orally by gavage on day 17 of pregnancy. Their offspring (62 females and 43 males) received 96 weekly oral doses of styrene oxide (100–150 mg/kg bw) in olive oil from four weeks until 120 weeks of age, at which time the experiment was terminated (estimated total doses, 2.5 g for females and 5.0 g for males). Groups of control rats received olive oil only. Of the treated progeny, 60 males and 42 females were alive at the time of appearance of the first tumour. A statistically significant increase in the incidence of forestomach tumours was observed in the styrene oxide-treated progeny of both sexes:

[1]The Working Group was aware of studies in progress in mice and rats by oral administration (IARC, 1982b).

papillomas in 9/102; carcinoma *in situ* in 10/102; and carcinomas in 26/102. In the progeny of controls, the incidence of forestomach tumours was: papillomas, 2/104; and carcinomas, 1/104. No difference between treated and control groups was observed in the incidence of tumours occurring at other sites (Ponomarkov *et al*., 1984). [The Working Group could not evaluate the effects of the prenatal treatment, since no appropriate control group was available.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The toxicity of styrene oxide has recently been reviewed (International Programme on Chemical Safety, 1983).

In rats, the oral LD_{50} of styrene oxide was reported to be 4290 mg/kg bw (Smyth *et al*., 1954) or 3000 mg/kg bw (Weil *et al*., 1963); the intraperitoneal LD_{50} was 460–610 mg/kg bw (Ohtsuji & Ikeda, 1971). The LD_{50} by skin application in rabbits was reported as 1184 mg/kg bw (Smyth *et al*., 1954) or 930 mg/kg bw (Weil *et al*., 1963). Inhalation of 4900 mg/m^3 (1000 ppm) in air killed 2/6 rats within four hours (Weil *et al*., 1963).

Styrene oxide causes corneal injury in rabbits (Weil *et al*., 1963); even dilutions as low as 1% cause eye irritation (Hine & Rowe, 1963). Intradermal injections sensitized the skin of guinea-pigs (Weil *et al*., 1963).

One intraperitoneal dose of 375 mg/kg bw styrene oxide caused a decrease in the activities of rat-liver mixed-function oxidases and in cytochrome P-450 content (Parkki *et al*., 1976). Styrene oxide decreased rat-liver glutathione content *in vivo* at doses of 50 and 200 mg/kg bw (Marniemi *et al*., 1977).

Effects on reproduction and prenatal toxicity

Doses of 0, 0.5, 1, 2, 2.5 or 5 μmol/egg styrene oxide (purum grade) dissolved in ethanol were injected into the air space of groups of 10–20 White Leghorn SK 12 chicken eggs on day three of incubation. Embryos were examined on day 14 of incubation. Concentrations above 0.1 μmol [data for this dose were not presented] reduced embryonic viability (LD_{50}, 1.5 μmol/egg), and malformations were observed in 7% of the treated eggs and 0% of control eggs. The lowest effective dose that produced malformations was 0.5 μmol/egg (Vainio *et al*., 1977). No dose-response relationship was observed.

Doses of 0.8 μmol/egg styrene oxide (purity, 97%) dissolved in vegetable oil were injected into the air space of White-Leghorn 'mittari' and SK 12 chicken eggs on day three of incubation. In additional groups, 0.1 μmol trichloropropylene oxide (TCPO), an inhibitor of epoxide hydrolase, was injected simultaneously with styrene oxide as a check on the effects of metabolism on embryotoxicity. Embryos were examined on day 14 of incubation. Styrene oxide treatment alone resulted in embryolethality and malformations; addition of TCPO to the styrene oxide treatment augmented these effects (Kankaanpää *et al*., 1979).

Groups of 23–24 New Zealand white rabbits were exposed by inhalation to 0, 15 or 50 ppm (74 or 245 mg/m^3) (measured concentration of 14.6 or 51 ppm) styrene oxide (purity, 99%) vapour for seven hours per day on days 1–24 of gestation. Foetuses were examined on

day 30. Exposure to styrene oxide resulted in maternal toxicity (increased mortality, decreased food consumption and weight gain) and increased the frequency of resorptions. Maternal mortality was 1/23, 4/24 and 19/24, and the resorption rates were 0.25, 0.93 and 1.5 per litter in the control, low- and high-dose groups, respectively (Hardin *et al.,* 1981; Sikov *et al.,* 1981, 1984).

Six groups of at least 31 Sprague-Dawley rats were exposed by inhalation to 100 ppm (490 mg/m^3) or 300 ppm (1470 mg/m^3) styrene oxide (purity, 99%) vapour for seven hours per day either during a three-week pregestational period only, during a three-week pregestational period and through days 1–19 of gestation, or on gestation days 1–19 only. A control group was exposed to air during the whole period. Foetuses were examined on day 21. There was extensive mortality in rats that received prolonged exposure to 100 ppm; exposures at 300 ppm were discontinued after one day due to mortality. Maternal weight gain was reduced in all groups receiving 100 ppm. Gestational exposure decreased fecundity by increasing the preimplantation loss of embryos. Foetal weights and lengths were reduced, and the incidences of ossification defects of the sternebrae and occipital bones were increased by gestational exposure (Hardin *et al.,* 1981; Sikov *et al.,* 1981, 1986).

Absorption, distribution, excretion and metabolism

The metabolism and pharmacokinetics of styrene oxide have been reviewed by Leibman (1975) and Vainio *et al.* (1984a,b) (see Fig. 1).

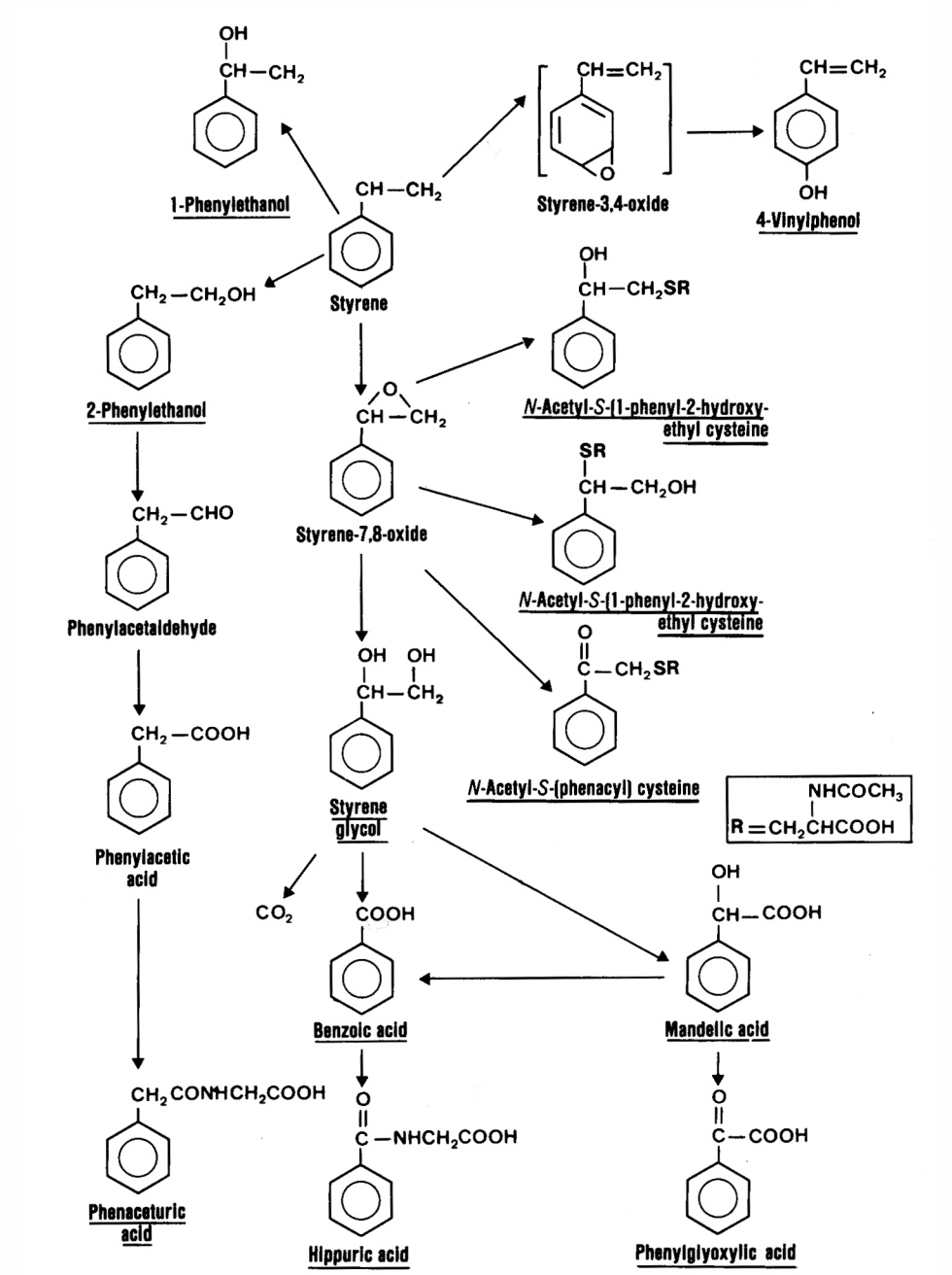
After mice received an intraperitoneal injection of styrene, the highest concentrations of styrene oxide were detected in the kidneys, subcutaneous adipose tissue and blood from one to five hours after injection (Nordqvist *et al.,* 1983; Löf *et al.,* 1984). Styrene oxide is a metabolite of styrene (Leibman & Ortiz, 1970; Norppa *et al.*, 1980) (see Fig. 1). It is also formed non-enzymatically through catalysis by oxyhaemoglobin (Belvedere *et al.,* 1983).

Styrene oxide is hydrolysed *in vitro* to styrene glycol (phenylethylene glycol) by microsomal epoxide hydrolase from the liver, kidneys, intestine, lungs and skin of several mammalian species (Oesch, 1973).

The biotransformation of styrene oxide to styrene glycol was stimulated by pretreatment of rats with phenobarbital or 3-methylcholanthrene (Oesch *et al.,* 1971); further metabolism of styrene glycol to mandelic acid was not stimulated (Ohtsuji & Ikeda, 1971). Isolated, perfused rat liver rapidly metabolized styrene oxide to styrene glycol, mandelic acid and glutathione conjugates (Ryan & Bend, 1977; Steele *et al.,* 1981). Microsomal conjugation of styrene oxide with glutathione yields about 60% *S*-(1-phenyl-2-hydroxyethyl)glutathione and 40% *S*-(2-phenyl-2-hydroxyethyl)glutathione (Pachecka *et al.,* 1979).

The main route of excretion of styrene oxide metabolites in animals is *via* the kidney; in rabbits, about 80% of a single oral dose was excreted in the urine (James & White, 1967). Acidic urinary metabolites of styrene oxide derived from glutathione conjugates are species dependent. In rats, the only products detected are the mercapturic acids. In guinea-pigs, the major bivalent sulphur acids are the corresponding mercaptoacetic acids, together with mercaptolactic and mercaptopyruvic and mercapturic acids. Some reduction of styrene oxide to styrene may occur in rats and guinea-pigs, with subsequent formation of the dihydrodiol, 3,4-dihydroxy-3,4-dihydro-1-vinylbenzene, which has been found as a urinary metabolite of both styrene and styrene oxide (Nakatsu *et al.,* 1983).

Fig. 1. Metabolic pathways of styrene oxide[a]



[a]From Vainio *et al*. (1984a); underlined metabolites are excreted in urine.

[7-^{3}H]-Styrene oxide injected intraperitoneally or incubated *in vitro* binds covalently to microsomes, protein and nucleic-acid fractions of rat liver (Marniemi *et al*., 1977); in perfused rat liver, binding to RNA and DNA is also detected (Van Anda *et al.,* 1979). When styrene oxide was reacted with polyamino acids *in vitro,* preferential binding to polycysteine was noted (Hemminki, 1983). Reaction products of styrene oxide with nucleosides were identified as 7-alkylguanine and 3-alkylcytosine (Hemminki *et al.,* 1980; Sugiura & Goto, 1981); adducts at N^2- and O^6-guanosine have been described (Hemminki & Hesso, 1984). Esterification of phosphate groups in thymidine monophosphate has been noted (Hemminki & Suni, 1984).

Mutagenicity and other short-term tests (see also 'Appendix: Activity Profiles for Short-term Tests', p. 341)

Styrene oxide was mutagenic to *Salmonella typhimurium* TA1535 and TA100 in the absence of an exogenous metabolic system (S9); it was not mutagenic to strains TA1537, TA1538 or TA98 in the presence or absence of S9 (Milvy & Garro, 1976; Vainio *et al.,* 1976; Stoltz & Withey, 1977; Loprieno *et al.,* 1978; de Meester *et al.,* 1978; Sugiura *et al.,* 1978a; Ueno *et al.,* 1978; Wade *et al.,* 1978; Watabe *et al.,* 1978; Busk, 1979a; Yoshikawa *et al.,* 1980; De Flora, 1981; de Meester *et al.,* 1981; Turchi *et al.,* 1981; Glatt *et al.,* 1983). Activity was reduced by glutathione and S9 (de Meester *et al.,* 1978; Busk, 1979b; Yoshikawa *et al*., 1980; De Flora, 1981). The (R) enantiomer of styrene oxide is more mutagenic to *S. typhimurium* TA100 than is the (S) enantiomer (Pagano *et al.,* 1982). Mutations were also induced by styrene oxide in *Escherichia coli* WP2 in the absence of an exogenous metabolic system (Sugiura *et al*., 1978b; Sugiura & Goto, 1981).

Styrene oxide was mutagenic to *Klebsiella pneumoniae* (Voogd *et al.,* 1981). It induced forward mutations in *Schizosaccharomyces pombe in vitro* and in the host-mediated assay in which mice were given oral doses of 100 mg/kg bw. [The authors report the latter as a negative result; however, the data indicate a positive effect.] The compound induced mitotic gene conversion in *Saccharomyces cerevisiae in vitro,* and in the host-mediated assay in which mice were given oral doses of 100 mg/kg bw (Loprieno *et al.,* 1976).

Chromosomal aberrations and micronucleated cells were observed in root-tip cells of *Allium cepa* treated with styrene oxide (Linnainmaa *et al.,* 1978a,b).

Sex-linked recessive lethal mutations were observed in *Drosophila melanogaster* exposed to 200 ppm (980 mg/m^3) styrene oxide vapour for six hours per day for four days, or fed a 200 mg/kg solution in 1% sucrose for 24 hours (Donner *et al.,* 1979).

Treatment of a primary culture of rat hepatocytes with 0.3 mM [36 µg/ml] styrene oxide induced DNA single-strand breaks, as evaluated by alkaline elution (Sina *et al.,* 1983). Unscheduled DNA synthesis was induced in a human heteroploid cell line following exposure to styrene oxide in the absence of an exogenous metabolic system (Loprieno *et al.,* 1978). It was reported in an abstract that styrene oxide induces unscheduled DNA synthesis in human amniotic cells (Audette *et al.,* 1979).

The compound induced forward mutations (HGPRT locus) in Chinese hamster V79 cells (Loprieno *et al.,* 1976; Bonatti *et al.,* 1978; Loprieno *et al.,* 1978; Beije & Jenssen, 1982). Perfusion of styrene oxide through isolated liver abolished its mutagenic effect to V79 cells (Beije & Jenssen, 1982). It was positive in the mouse-lymphoma L5178Y assay (TK$^{+/-}$); activity was reduced by addition of S9 (Amacher & Turner, 1982).

Styrene oxide induces chromosomal aberrations in Chinese hamster V79 cells (Turchi *et al*., 1981) and in human lymphocytes (Fabry *et al*., 1978; Linnainmaa *et al.,* 1978a,b; Norppa *et al.* 1981). It induces micronuclei in Chinese hamster V79 cells (Turchi *et al.,* 1981) and cultured human lymphocytes (Linnainmaa *et al.,* 1978a,b), and sister chromatid exchanges in Chinese hamster ovary cells (de Raat, 1978) and cultured human lymphocytes (Norppa *et al*. 1981).

Styrene oxide injected intraperitoneally into male mice at 1.8–7.0 mmol/kg bw induced single-strand breaks in the DNA of liver, lung, kidney, testis and brain (Walles & Orsén, 1983). It did not induce chromosomal aberrations or micronuclei in the bone-marrow cells of BALB/c mice treated intraperitoneally with 250 mg/kg bw, or dominant lethal mutations or translocations in meiotic male germ cells of BALB/c mice (Fabry *et al*., 1978).

Styrene oxide was negative in a micronucleus test with Chinese hamsters given a single intraperitoneal injection of 250 mg/kg bw (Penttilä *et al.,* 1980).

No increase in the incidence of chromosomal aberrations or sister chromatid exchanges was observed in bone-marrow cells of male Chinese hamsters treated by inhalation with 25, 50, 75 or 100 ppm (122, 245, 368 or 4900 mg/m^3) styrene oxide vapour for two, four and 21 (25 ppm only) days (Norppa *et al.,* 1979). In a preliminary report, inhalation exposure of mice to 50 ppm (245 mg/m^3) styrene oxide vapour induced a slight increase in sister chromatid exchanges in regenerating liver cells and alveolar macrophages, but not in bone-marrow cells (Conner *et al.,* 1982).

Styrene oxide induced chromosomal aberrations in the bone-marrow cells of male CD-1 mice treated by gavage with 50, 500 or 1000 mg/kg bw (Loprieno *et al.,* 1978).

(*b*) *Humans*

Toxic effects

Acute exposure to styrene oxide causes skin and eye irritation and skin sensitization (Hine *et al.,* 1981).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

No data were available on the absorption, distribution, excretion and metabolism after human exposure to styrene oxide alone. However, the main urinary excretion products of styrene include mandelic acid and phenylglyoxylic acid, both of which involve styrene oxide as an intermediate (Leibman, 1975; Vainio *et al.,* 1984b). Low concentrations of styrene oxide (0.05 μmol/L) have been detected in the venous blood of four workers exposed to styrene of unspecified purity (Wigaeus *et al.,* 1983).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Commercial production of styrene oxide was first reported in 1974. This compound is used as a reactive plasticizer and diluent for epoxy resins and as a chemical intermediate. It has been found in low concentrations in the air of workplace environments where styrene was used, and has been detected in the blood of workers exposed to styrene.

4.2 Experimental data

Styrene oxide was tested by intragastric intubation in rats of one strain and induced squamous-cell carcinomas and papillomas of the forestomach. Prenatal exposure followed by postnatal oral administration of styrene oxide to rats of another strain also produced squamous-cell carcinomas and increased the incidence of papillomas of the forestomach. No increase in the incidence of skin tumours was observed in mice of two strains following topical application of styrene oxide.

Exposure of rats and rabbits by inhalation to styrene oxide vapour at maternally toxic doses did not result in malformations; however, there was an increase in preimplantation losses and ossification defects in rats and an increased resorption frequency in rabbits.

Styrene oxide was mutagenic to bacteria, yeast and insects; it induced chromosomal aberrations and micronuclei in plants. The compound was mutagenic to mammalian cells *in vitro;* it induced DNA damage in mammalian cells both *in vivo* and *in vitro,* chromosomal

Overall assessment of data from short-term tests: styrene oxide[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/Green plants		+	+	
Insects		+		
Mammalian cells (*in vitro*)	+	+	+	
Mammals (*in vivo*)	+		?	
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: no data

[a]The groups into which the table is divided and the symbols '+' and '?' are defined in the Preamble; the degrees of evidence are also defined.

aberrations and sister chromatid exchanges *in vitro.* In several studies in mice and hamsters *in vivo,* no dominant lethal mutations, chromosomal aberrations, micronuclei or sister chromatid exchanges were induced; however, in one study in mice, styrene oxide induced chromosomal aberrations.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of styrene oxide to humans was available to the Working Group.

4.4 Evaluation

There is *sufficient evidence*[1] for the carcinogenicity of styrene oxide to experimental animals.

No data on the carcinogenicity of styrene oxide to humans were available to the Working Group.

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[1] In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans.

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STYRENE-7,8-OXIDE

This substance was considered by previous Working Groups, in February 1976 (IARC, 1976), February 1978 (IARC, 1979) and June 1984 (IARC, 1985). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 96-09-3

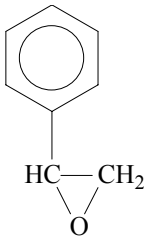
Replaced CAS Reg. No.: 62497-63-6

Chem. Abstr. Name: Phenyloxirane

IUPAC Systematic Name: (Epoxyethyl)benzene

Synonyms: 1,2-Epoxyethylbenzene; 1,2-epoxy-1-phenylethane; epoxystyrene; α,ß-epoxystyrene; phenethylene oxide; 1-phenyl-1,2-epoxyethane; phenylethylene oxide; 2-phenyloxirane; styrene epoxide; styrene oxide; styryl oxide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_8H_8O Relative molecular mass: 120.15

1.1.3 *Chemical and physical properties of the pure substance*

From Union Carbide Corp. (1984) and Rhone-Poulenc Chimie (1985), unless otherwise specified.

(a) *Description*: Colourless liquid

(b) *Boiling-point*: 194.1 ºC

(c) *Freezing-point*: -36.7 ºC

(d) *Density*: 1.050-1.054 at 20 ºC/4 ºC

(e) *Spectroscopy data*: Infrared, ultraviolet [2303], nuclear magnetic resonance and mass spectral data have been reported (Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993a).

(f) *Solubility*: Slightly soluble in water (3 g/L at 25 ºC); soluble in acetone, benzene, carbon tetrachloride, heptane and methanol

(g) *Volatility*: Vapour pressure, < 1 mm Hg [133 Pa] at 20 ºC

(h) *Stability*: Flash-point, 80-82 ºC (open cup); polymerizes exothermically and reacts violently with water in the presence of catalysts (acids, bases, certain salts)

(i) *Octanol-water partition coefficient* (*P*): log P, 1.61 (Sangster, 1989)

(j) Conversion factor: $mg/m^3 = 4.91 \times ppm$[a]

1.1.4 *Technical products and impurities*

Styrene-7,8-oxide exists in two optical isomers (see section 4), and the commercial product is a racemic mixture. Typical product specifications are for 99% minimal purity and 0.1-0.2% maximal water content (Union Carbide Corp., 1984; Rhone-Poulenc Chimie, 1985).

1.1.5 *Analysis*

Styrene-7,8-oxide can be determined in air by gas chromatography with mass spectrometry or flame ionization detection. The sample is collected on solid sorbent and desorbed thermally or with ethyl acetate (Pellizzari *et al*., 1976; Taylor, 1979; Stampfer & Hermes, 1981). Detection limits as low as 2 ng/m^3 have been reported (Krost *et al*., 1982).

1.2 Production and use

1.2.1 *Production*

Styrene-7,8-oxide is produced commercially by the reaction of styrene with chlorine and water to form styrene chlorohydrin, followed by cyclization with aqueous base to produce styrene-7,8-oxide. It is also prepared by epoxidation of styrene with peroxyacetic acid (US National Library of Medicine, 1993a).

Information available in 1991 indicated that styrene-7,8-oxide was produced by three companies in Japan and one in the USA (Chemical Information Services Ltd, 1991).

1.2.2 *Use*

Styrene-7,8-oxide is used as a chemical intermediate in several processes. Hydrogenation yields 2-phenylethanol, which is also known as 'oil of roses', a widely used perfume base. Esters useful in fragrance applications can be made by reacting styrene-7,8-

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[a] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming normal temperature (25 ºC) and pressure (101.3 kPa)

oxide with carboxylic acids. Reaction of styrene-7,8-oxide with ethanolamine yields an intermediate used in the synthesis of tetramisole, a commercial anthelmintic. The low viscosity of styrene-7,8-oxide and its reactivity have led to its use as a reactive diluent for epoxy resins. It is also reported to be used in cross-linked polyesters and polyurethanes. It is added in small quantities as a reactive acid scavenger to improve the stability of hydraulic fluids, chlorinated cleaning compositions, petroleum distillates, dielectric fluids and acid-sensitive polymers and copolymers. Styrene-7,8-oxide can be homopolymerized to poly(styrene glycols) and copolymerized with other epoxides. It is also used in adhesive formulations, as a polypropylene catalyst deactivator, to make graft copolymers of cotton, silk and wool, as a lubricant for acetal polymers and in sealant formulations based on silylated polyurethanes (Union Carbide Corp., 1984).

1.3 Occurrence

1.3.1 *Natural occurrence*

Styrene-7,8-oxide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 450 employees were potentially exposed to styrene-7,8-oxide in the USA (US National Institute for Occupational Safety and Health, 1993). Of this number 59% were estimated to be exposed to styrene-7,8-oxide and 41% to materials containing styrene-7,8-oxide. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Occupational exposure to styrene-7,8-oxide may occur because of its formation from styrene in industries where polyester resins with styrene are used when peroxides are added to the resin. In Finnish factories for producing boats, car parts and building materials from polyester-based reinforced plastics, the average styrene-7,8-oxide levels in personal air samples were found to be 0.04 ppm [0.20 mg/m^3] for hand lay-up and 0.12 ppm [0.59 mg/m^3] for spray application; the corresponding styrene levels were 133 and 130 ppm [567 and 554 mg/m^3] (Pfäffli *et al*., 1979). In a Norwegian factory where similar processes were used, styrene-7,8-oxide levels ranged from < 0.003 to 0.12 ppm [< 0.015-0.59 mg/m^3] and concurrent styrene levels from 17 to 289 ppm [72-1230 mg/m^3] (Fjeldstad *et al*., 1979). Similarly, in a boat manufacturing company in the USA, the mean styrene-7,8-oxide level was 0.14 mg/m^3 for the 19 workers most heavily exposed to styrene (mean, 64 mg/m^3) (Rappaport *et al*., 1991). Data obtained in 32 Finnish plants allow the rough calculation of a ratio of styrene-7,8-oxide to styrene of 1:1000 (Säämänen *et al*., 1993).

Acetophenone and benzaldehyde, oxidized products of styrene-7,8-oxide, were quantified in personal samples at mean levels of 0.47 and 0.48 ppm [2.3 and 2.4 mg/m^3], respectively, during spray application in Finland (Pfäffli *et al*., 1979).

1.3.3 *Water and sediments*

In a comprehensive survey of 4000 samples of wastewater taken from a broad range of industrial and publicly owned treatment works in the USA, styrene-7,8-oxide was identified

in one discharge from rubber processing at a level of 46.2 ppb [μg/L] (US National Library of Medicine, 1993a).

1.3.4 *Other*

Annual total air emissions of styrene-7,8-oxide in the USA, reported to the US Environmental Protection Agency by industrial facilities, were 464 kg in 1987 from two locations, 1050 kg in 1988 from six locations, 918 kg in 1989 from five locations, 1099 kg in 1990 from five locations and 760 kg in 1991 from five locations. Total releases to ambient water in 1987 were estimated to result in 353 kg (US National Library of Medicine, 1993b).

1.4 Regulations and guidelines

No regulations or guidelines have been established for occupational exposure to styrene-7,8-oxide (American Conference of Governmental Industrial Hygienists, 1993; ILO, 1993; UNEP, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 52 male and 52 female B6C3F_1 mice, seven weeks old, were administered 0 (control), 375 or 750 mg/kg bw styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified amounts of benzaldehyde and benzene) in corn oil by gastric intubation daily three times a week for 104 weeks. Three to four weeks after the last dose, all surviving animals were killed. There was a marked reduction in the survival of high-dose male and female mice, and body weights were reduced in both groups. Treatment resulted in a significant ($p < 0.001$) increase in the incidence of squamous-cell carcinoma of the forestomach in males at both dose levels (control, 0/51; low-dose, 16/51; high-dose, 15/52) and in females at the low dose (control, 0/51; low-dose, 10/50; high-dose, 3/51), and a significant increase in the incidence of squamous-cell papillomas at both dose levels in males (control, 2/51; low-dose, 22/51; high-dose, 8/52) and females (control, 0/51; low-dose, 14/50; high-dose, 17/51). The incidences of squamous-cell papillomas and carcinomas (combined) were: males—control, 2/51; low-dose, 37/51; high-dose, 21/52; and females—control, 0/51; low-dose, 24/50; high-dose, 20/51 ($p < 0.001$). Low-dose males had a significant increase in the incidence of hepatocellular tumours: 12/51 in controls; 28/52 in the low-dose group ($p < 0.001$; Fisher's exact test) (Lijinsky, 1986).

3.1.2 *Rat*

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, were administered 0 (control), 50 or 250 mg/kg bw styrene-7,8-oxide [purity unspecified] in olive oil by gastric intubation daily on four to five days per week for 52 weeks. Rats were kept until they died; the last death occurred 156 weeks after initial dosing. There was no effect of treatment on survival or body weight. Treatment resulted in a dose-dependent increase in the incidence of squamous-cell carcinoma of the forestomach in males (control, 0/40; low-dose, 11/40; high-dose, 30/40) and females (control, 0/40; low-dose, 8/40; high-dose, 33/40). The incidences of squamous-cell papilloma/acanthoma were: males—control, 0/40; low-dose, 3/40; high-dose, 9/40; and females—control, 0/40; low-dose, 3/40; high-dose, 5/40. The incidences of acanthosis and dysplasia of the forestomach epithelium were treatment-related. No increase in the incidence of tumours at other sites was found (Maltoni *et al*., 1979; Conti *et al*., 1988).

Groups of 52 male and 52 female Fischer 344/N rats, nine weeks old, were administered 0 (control), 275 or 550 mg/kg styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified amounts of benzaldehyde and benzene) in corn oil by gastric intubation daily three times a week for 104 weeks. The experiment was terminated at 107-108 weeks. Body weights and survival were reduced in high-dose males and females. Treatment resulted in a significant increase in the incidence of squamous-cell carcinoma of the forestomach in males (control, 0/52; low-dose, 35/52; high-dose, 43/51) and females (control, 0/52; low-dose, 21/52; high-dose, 24/52); the incidence of squamous-cell papilloma was also increased (males: 1/52, 23/52, 18/51; females: 0/52, 21/52, 24/52) in treated rats. The incidences of combined squamous-cell papilloma and carcinoma were: males—control, 1/52; low-dose, 50/52; high-dose, 50/51; and females—control, 0/52; low-dose, 46/52; high-dose, 50/52. No increase in the incidence of tumours at other sites was found (Lijinsky, 1986).

3.2 Prenatal exposure followed by postnatal oral administration

Rat: A group of 14 pregnant female BDIV inbred rats [age unspecified] was administered 200 mg/kg bw styrene-7,8-oxide (purity, 97%) in olive oil by gastric intubation on day 17 of gestation. Their offspring (62 females and 43 males) received 96 doses of styrene-7,8-oxide (100-150 mg/kg bw) in olive oil by gastric intubation once a week beginning at four weeks of age. The study was terminated at 120 weeks to give estimated total doses of 2.5 g for females and 5.0 g for males. Control groups of 49 male and 55 female rats with no prenatal exposure received olive oil alone. At the time of appearance of the first tumour, 60 female and 42 male progeny that had been treated with styrene-7,8-oxide were still alive. The incidences of forestomach tumours in control and treated groups were: papilloma—males, 0/49 *versus* 7/42 ($p < 0.003$); females, 2/55 *versus* 2/60 ($p > 0.05$); carcinoma *in situ*—males, 0/49 *versus* 4/42 ($p < 0.04$); females, 0/55 *versus* 6/60 ($p < 0.02$); carcinoma—males, 0/49 *versus* 10/42 ($p < 0.0002$); females, 1/55 *versus* 16/60 ($p < 0.0001$). Hyperplasia, dysplasia and hyperkeratosis of the forestomach were also reported in treated rats. There was no difference between treated and control groups in the incidence of tumours at other sites (Ponomarkov *et al*., 1984).

3.3 Skin application

Mouse: A group of 40 C3H mice [sex unspecified], 13 weeks old, received three weekly applications of a 5% solution of styrene-7,8-oxide in acetone [volume unspecified] on the clipped dorsal skin for life. No skin tumour was observed in the 17 mice that survived to 24 months. Another group of 40 C3H mice was similarly treated with a 10% solution of styrene-7,8-oxide in acetone: 18 mice survived to 12 months, and only two mice survived to 17 months. No skin tumour was observed (Weil *et al*., 1963). [The Working Group noted the incomplete reporting of the study.]

A group of 30 male Swiss ICR/Ha mice, eight weeks old, received three weekly applications of 100 mg of a 10% solution of styrene-7,8-oxide in benzene on clipped dorsal skin for life. The median survival time was 431 days. Three mice developed skin tumours, one of which was a squamous-cell carcinoma. Of 150 benzene-painted controls, 11 developed skin tumours, one of which was a squamous-cell carcinoma (Van Duuren *et al*., 1963). [The Working Group noted the potential carcinogenicity of the vehicle.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, excretion and metabolism

For a review of the metabolism and pharmacokinetics of styrene-7,8-oxide see the monograph on styrene.

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

(a) *Styrene-7,8-oxide*

In mice that received an intraperitoneal injection of styrene, the maximal concentrations of styrene-7,8-oxide were higher in subcutaneous adipose tissue than in the other tissues studied 1-5 h after injection (Nordquist *et al*., 1983; Löf *et al*., 1984).

The absorption and elimination of styrene-7,8-oxide were investigated in $CD2F_1$ mice after a single intraperitoneal injection of 200 mg/kg bw in corn oil (Bidoli *et al*., 1980). Styrene-7,8-oxide was rapidly absorbed, reaching a peak concentration in blood of 40 ± 7 μg/mL at 7 min, after which it rapidly disappeared; at 60 min, it was no longer detectable. The area under the curve for the time course of the blood concentration of styrene-7,8-oxide was 329 min × μg/g.

The pharmacokinetics of styrene-7,8-oxide in male Fischer 344 rats was studied after oral administration of 275 and 550 mg/kg bw (Langvardt & Nolan, 1991). Wide variation was seen in the measured blood concentrations, which ranged from 0.27 to 8.84 μg/mL in animals given the low dose and from 2.1 to 32.4 μg/mL in those given the high dose. The

areas under the curve for the time course of the blood concentration of styrene-7,8-oxide were 47 and 286 min × μg/g.

The uptake, distribution and elimination of styrene-7,8-oxide were investigated in Sprague-Dawley rats and B6C3F$_1$ mice after intraperitoneal and oral administration of 200 mg/kg bw. Styrene-7,8-oxide was rapidly absorbed, reaching a peak concentration within 15 min. The blood concentrations varied widely between animals after oral administration. The areas under the curve for the time course of the blood concentration of styrene-7,8-oxide after intraperitoneal and oral administration were 18 and 0.76 h × μg/mL in rats and 12 and 0.01 h × μg/mL in mice, respectively. The significantly reduced bioavailability of styrene-7,8-oxide after oral administration was due to hydrolysis in the acidic environment of the stomach (Kessler *et al*., 1992), as indicated by the finding of acid-catalysed hydrolysis of styrene-7,8-oxide *in vitro* (Ross *et al*., 1982).

A physiological pharmacokinetic model was developed to describe the disposition and metabolism of styrene and styrene-7,8-oxide in mouse, rat and man (Csanády *et al*., 1994) after inhalation or intravenous, oral or intraperitoneal administration of styrene, and after intravenous, oral or intraperitoneal administration of styrene-7,8-oxide. The model includes oxidation of styrene to styrene-7,8-oxide, the intracellular first-pass hydrolysis of styrene-7,8-oxide catalysed by epoxide hydrolase and the conjugation of styrene-7,8-oxide with glutathione. Conjugation is described by an ordered sequential 'ping-pong' mechanism between glutathione, styrene-7,8-oxide and glutathione *S*-transferase. The model was validated with data sets from a number of laboratories on the pharmacokinetics of styrene and styrene-7,8-oxide in rodents and man. The effects of alveolar ventilation and the blood:air partition coefficient of styrene on the pharmacokinetics of styrene and styrene-7,8-oxide were investigated by sensitivity analysis. The sensitivity coefficients calculated for steady-state exposure to styrene at 500 ppm [2130 mg/m^3] indicated that small changes in the balance of production and elimination could cause drastic changes in the body burden of styrene-7,8-oxide in mice but not in rats or humans. These findings might explain the greater mortality among mice exposed to 250 and 500 ppm [1065 and 2130 mg/m^3] styrene (Morgan *et al*., 1993).

Styrene-7,8-oxide is the metabolite of styrene that is catalysed by the cytochrome P450 monooxygenase system and non-enzymatically by oxyhaemoglobin (Belvedere *et al*., 1983). Further metabolic reactions are catalysed by epoxide hydrolase and glutathione *S*-trans-ferase. When human cytosolic and microsomal epoxide hydrolases were assayed with styrene-7,8-oxide, the microsomal activity was greater than the cytosolic activity (Schladt *et al*., 1988). Human liver glutathione *S*-transferase cytosolic fractions occur in two forms, μ and α, of which the μ form was more active with styrene-7,8-oxide, with a K_m of 4.9 mmol/L and a V_{max} of 22 nmol/mg per min (Pacifici *et al*., 1987). About one-half of individuals in many Caucasian populations lack this enzyme (Warholm *et al*., 1981). Glutathione *S*-transferase and epoxide hydrolase activities were detected in many fetal tissues (Pacifici & Rane, 1982), and the α and π forms of glutathione *S*-transferase are present in fetal liver (Pacifici *et al*., 1988).

The enzymes that metabolize styrene-7,8-oxide are stereoselective, in that the S enantiomer is favoured over the R in subsequent hydrolysis by epoxide hydrolase (Watabe *et al*., 1981). In contrast, glutathione *S*-transferase, including the μ form, favours the R isomer

(Hiratsuka *et al*., 1989). The R forms were substituted to C7 and the S forms to C8 (Dostal *et al*., 1986).

Isolated, perfused rat liver rapidly metabolized styrene-7,8-oxide to styrene glycol, mandelic acid and glutathione conjugates (Ryan & Bend, 1977; Steele *et al*., 1981). Microsomal conjugation of styrene-7,8-oxide with glutathione yielded about 60% *S*-(1-phenyl-2-hydroxyethyl)glutathione and 40% *S*-(2-phenyl-2-hydroxyethyl)glutathione (Pachecka *et al*., 1979). (See the monograph on styrene for further description of styrene-7,8-oxide metabolism.)

The main route of excretion of styrene-7,8-oxide metabolites in animals is *via* the kidney: in rabbits, about 80% of a single oral dose was excreted in the urine (James & White, 1967). Acidic urinary metabolites of styrene-7,8-oxide derived from glutathione conjugates are species dependent: in rats, the only products detected are mercapturic acids; in guinea-pigs, the major bivalent sulfur acids are the corresponding mercaptoacetic acids, together with mercaptolactic and mercaptopyruvic and mercapturic acids. 3,4-Dihydroxy-3,4-dihydro-1-vinylbenzene has been reported as a urinary metabolite of both styrene and styrene-7,8-oxide in rats and guinea-pigs (Nakatsu *et al*., 1983).

(b) *Protein adducts*

In vitro, styrene-7,8-oxide bound to histidine in human haemoglobin (Kaur *et al*., 1989) but predominantly to cysteine in human plasma proteins (Hemminki, 1986). It bound to polyamino acids in the order: polycysteine >> polyhistidine > polylysine > polyserine (Hemminki, 1983). Cysteine alkylation was determined following intraperitoneal administration of styrene-7,8-oxide to rats (Rappaport *et al*., 1993).

Covalent binding to plasma proteins and haemoglobin were determined in male mice [strain unspecified] after intraperitoneal administration of [7-^{14}C]styrene and [7-^{3}H]-styrene-7,8-oxide. A dose-dependent increase in alkylated plasma proteins was seen 5 h after injection of 0.12-4.9 mmol/kg bw styrene or 2 h after injection of 0.12-2.4 mmol/kg bw styrene or 0.037-1.1 mmol/kg bw styrene-7,8-oxide. The plasma-protein binding ratio of styrene-7,8-oxide to styrene increased with dose, a result that is consistent with the saturable metabolism of styrene. In contrast, binding to haemoglobin 2 h after injection of 1.1-4.9 mmol/kg bw styrene was proportionally higher at high doses. One explanation given by the authors is an increased importance of metabolic activation of styrene by erythrocytes when a higher proportion of styrene escapes the hepatic metabolizing enzymes. Following administration of 0.037-1.1 mmol/kg bw styrene-7,8-oxide, proportionally greater binding to plasma proteins was observed at the highest dose (Byfält Nordqvist *et al*., 1985).

In mice treated intraperitoneally with styrene-7,8-oxide at 50-250 mg/kg bw, a disproportionate increase in binding was seen at higher dose levels. A lesser but similar effect was seen in rats, which showed an about three-fold lower adduct level at equivalent doses of styrene-7,8-oxide. In mice administered styrene at the same doses, about 5% of styrene was available as the oxide (Osterman Golkar, 1992).

Female Wistar rats treated intraperitoneally with styrene-7,8-oxide at 83-833 μmol/kg bw had haemoglobin carboxylic acid esters of styrene-7,8-oxide, the level of which increased with dose (disproportionately at higher doses). The lowest dose (83 μmol/kg bw)

resulted in an adduct level of 16.7 pmol/g globin, and 833 μmol/kg bw yielded 724 pmol/g globin (Sepai *et al*., 1993).

A similar study on covalent binding of styrene and styrene-7,8-oxide to albumin and haemoglobin was performed in Sprague-Dawley rats. Linear relationships were observed between adduct levels and intraperitoneal doses of 0.5-3 mmol/kg bw styrene and 0.1-1 mmol/kg bw styrene-7,8-oxide. Comparison of the slopes revealed a much greater production of protein adducts following administration of styrene-7,8-oxide, the slope derived for styrene being only 2% of that for styrene-7,8-oxide (Rappaport *et al*., 1993).

4.1.3 *Comparison of humans and animals*

Pieces of human liver from five accident victims selected for organ transplantation were obtained through the Nashville (USA) regional organ procurement agency. No information was available on the donors, other than that the livers were free of debilitating diseases, such as human immunodeficiency viral infection and hepatitis A and B. The activities of cytochrome P450 monooxygenase and microsomal and cytosolic forms of epoxide hydrolase and glutathione *S*-transferase were then compared in the livers of humans, Fischer 344 and Sprague-Dawley rats and $B6C3F_1$ mice (Mendrala *et al*., 1993). The affinities of the monooxygenases (inverse K_m values) were essentially similar: 0.09 mmol in humans and 0.05 mmol in mice not pretreated with styrene. The V_{max} values were similar in rats and mice (9.3-13 nmol/mg protein per min) but were lower in the five human samples (2.1 nmol/mg per min). The K_m values for epoxide hydrolase were low in humans (0.01 mmol), inter-mediate in rats (0.13-0.23 mmol) and high in mice (0.74 mmol); the V_{max} values did not differ between the species. Humans apparently had the lowest glutathione *S*-transferase activity towards styrene-7,8-oxide.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Human plasma α_1-proteinase inhibitor was inactivated *in vitro* by styrene-7,8-oxide (Ansari *et al*., 1988a). Administration of styrene-7,8-oxide together with acrolein or pyruvic aldehyde caused greater inhibition than any compound alone (Ansari *et al*., 1988b). Styrene-7,8-oxide inhibited the activity of glutathione *S*-transferase π isolated from human erythrocytes to about one-half at a concentration of 2 nmol (Ansari *et al*., 1987).

Styrene-7,8-oxide causes corneal injury in rabbits (Weil *et al*., 1963); even dilutions as low as 1% cause eye irritation (Hine & Rowe, 1980). Intradermal injections sensitized the skin of guinea-pigs (Weil *et al*., 1963).

One intraperitoneal dose of 375 mg/kg bw styrene-7,8-oxide decreased the rat-liver mixed-function oxidase activity for certain substrates and in total cytochrome P450 content (Parkki *et al*., 1976). Styrene-7,8-oxide decreased the glutathione content of rat liver *in vivo* at doses of 50 and 200 mg/kg bw (Marniemi *et al*., 1977).

Styrene-7,8-oxide administered intraperitoneally to inbred male albino rats at doses of 25 and 50 mg/kg bw increased the levels of noradrenaline in the cerebral cortex, increased the activity of 5-hydroxytryptamine and decreased the activity of monoamine oxidase in several regions of the brain (Husain *et al*., 1985). In similar experiments, styrene-7,8-oxide treatment increased the total number of dopamine receptors (Zaidi *et al*., 1985). Styrene-7,8-oxide administered as single intraperitoneal doses of 100-400 mg/kg bw to Sprague-Dawley rats decreased the level of glutathione in the brain (Trenga *et al*., 1991). The effect was potentiated by arylamide, which caused necrosis of cerebellar granule cells and some small neurones of the cerebral cortex (Beiswanger *et al*., 1993).

Cell proliferation (as measured by the proportion of nuclei labelled with 5-bromo-2′-deoxyuridine delivered from a subcutaneously implanted osmotic pump during the last 24 h of the experiment) was increased in three regions of the forestomach of male Fischer 344 rats after gavage administration of styrene-7,8-oxide three times per week for four weeks. The doses used were 0, 137, 275 and 550 mg/kg bw. Only marginal morphological changes were observed occasionally (Cantoreggi *et al*., 1993).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Only one study of the reproductive toxicity of styrene-7,8-oxide in mammals has been published (Sikov *et al*. 1986). Six groups of at least 31 Wistar rats were exposed by inhalation (whole body) to 100 ppm [490 mg/m^3] or 300 ppm [1470 mg/m^3] styrene-7,8-oxide (purity, 99%) vapour for 7 h per day either during a three-week (five days/week) pregestational period, during a three-week (five days/week) pregestational period and through days 1-19 of gestation, or on gestational days 1-19 only. A control group was exposed to air during the whole period. Fetuses were examined on day 21. There was extensive mortality among rats that received prolonged exposure to 100 ppm; exposure to 300 ppm was discontinued after one day because of mortality. Maternal weight gain was reduced in all groups receiving 100 ppm. Exposure only prior to mating had no effect on mating or fertility. Gestational exposure decreased the number of animals pregnant at term by increasing preimplantation loss of embryos; fetal weights and lengths were reduced, and the incidences of retarded ossification of the sternebrae and occipital bones were increased. In the same study, groups of 23-24 New Zealand white rabbits were exposed by inhalation to 0, 15 or 50 ppm [74 or 245 mg/m^3] (measured concentrations, 14.6 and 51 ppm) styrene-7,8-oxide (purity, 99%) vapour for 7 h per day on days 1-24 of gestation. Fetuses were examined on day 30. Maternal toxicity was observed at the highest dose only, resulting in increased mortality (19/24 *versus* 1/23 in controls and 4/24 at 15 ppm) and decreased food consumption and weight gain. There was no effect on the proportion pregnant at term, i.e. there was no marked preimplantation loss, but there was an increase in postimplantation loss, with 0.25, 0.93 and 1.5 resorptions per litter in the control, low- and high-dose groups,

respectively. There was no effect on fetal weight, and no increase in the incidence of malformations was observed in either rats or rabbits.

4.4 Genetic and related effects

4.4.1 *Humans*

No published data on the effects of exposure of humans to styrene-7,8-oxide alone were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 1 and Appendices 1 and 2)

(a) *DNA adducts*

A comprehensive review of DNA adduct formation with styrene-7,8-oxide is available (Phillips & Farmer, 1994).

The relative yields of alkylated deoxynucleosides in DNA in aqueous buffer were deoxyguanosine > deoxycytidine > deoxyadenosine > thymidine, the dominant product being 7-alkylguanine (Savela *et al*., 1986). When radioactive styrene-7,8-oxide was reacted with double- and single-stranded DNA, the latter produced more adducts, the majority (54%) of which were 7-guanine adducts, representing similar proportions of α and ß isomers (Vodička & Hemminki, 1988a). Depurination of 7-alkyldeoxyguanosine derivatives of styrene-7,8-oxide occurred at the same rate as for 7-methyldeoxyguanosine, while depurination of 7-alkylguanine was 15 times slower in single-stranded DNA and 55 times slower in double-stranded DNA (Vodička & Hemminki, 1988b).

7-Alkylguanine adducts of styrene-7,8-oxide were demonstrated in five organs of mice after intraperitoneal injection of styrene-7,8-oxide (Byfält Nordqvist *et al*., 1985).

Using ^{32}P-postlabelling methods (Liu *et al*., 1988) with mammalian cells in culture, Pongracz *et al*. (1989) detected six adducts and identified two isomers of O^6-modified deoxyguanosines, *O*^{6}-(2-hydroxy-2-phenylethyl)-2′-deoxyguanosine-3′,5′-bisphosphate and *O*^{6}-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′,5′-bisphosphate. Hemminki *et al*. (1990) studied the stability of the deoxyguanosine 3'-monophosphate 7-alkylation products for postlabelling, but considerable lability of the 7-guanine adducts was observed. Further ^{32}P-postlabelling was performed using N7, N^2 and O^6 adducts of styrene-7,8-oxide (Vodicka & Hemminki, 1991). No phosphorylation products of N7 adducts were seen, while one of the two diastereomeric N^2 adducts was labelled with 20% efficiency, two of the three O^6 adducts with 5% efficiency and the third with 10% labelling efficiency, suggesting stereoselectivity of the kinase reaction.

Pongracz *et al*. (1992) detected six adducts of styrene-7,8-oxide in calf thymus DNA by ^{32}P-postlabelling, the N^2-guanosine derivatives being the major products. Combination of mass spectrometry with the postlabelling assay allowed identification of three new hydrophobic bis-substituted adducts representing N1, N^2 and N^2, O^6 modifications (Kaur *et al*., 1993). These modifications are unlikely to occur *in vivo* (Phillips & Farmer, 1994).

Radiolabelled [7-^{3}H]styrene-7,8-oxide was used to search for adducts in different parts of the gastrointestinal tract and liver of rats and mice *in vivo*. Covalent binding of

styrene-7,8-oxide occurred below the limit of detection in all tissues (Cantoreggi & Lutz, 1992). [The Working Group calculated that the maximal possible covalent binding index—(pmol adduct/mol DNA nucleotide)/(mmol chemical/kg bw)—was < 0.6 for mouse liver DNA 2 h after intraperitoneal injection.] In further studies with higher concentrations of styrene-7,8-oxide, binding to DNA in rat forestomach was detected, the covalent binding index being 1.0 (Lutz *et al*., 1993).

(b) *Mutation and allied effects*

Styrene-7,8-oxide induced SOS repair and reverse mutations in *Salmonella typhimurium* and *Escherichia coli*. Forward mutations were also induced in *S. typhimurium* and *Klebsiella pneumoniae*. The R enantiomer of styrene-7,8-oxide was slightly more mutagenic in *S. typhimurium* TA100 than the respective S enantiomer (Seiler, 1990; Sinsheimer *et al*., 1993). The sensitivity of *S. typhimurium* TA100 mutants to DL-1,2,4-triazole-3-alanine indicated that > 95% of the mutants were *his* locus revertants, the remainder being suppressors (Einistö *et al*., 1993).

Forward mutations and gene conversion were induced in yeasts, both *in vitro* and in the mouse host peritoneal assay. Chromosomal aberrations and micronuclei were induced in the plant, *Allium cepa*.

Sex-linked recessive lethal mutations were induced by styrene-7,8-oxide in *Drosophila melanogaster* in a single study.

In cultured mammalian cells, styrene-7,8-oxide induced DNA single-strand breaks (but not double-strand breaks or cross-links), mutations at the *hprt* and *tk* loci, sister chromatid exchange, micronuclei and chromosomal aberrations. Styrene-7,8-oxide tested in human cells in culture induced sister chromatid exchange, micronuclei and chromosomal aberrations. All of the reports of significant increases in the frequencies of sister chromatid exchange and chromosomal aberrations relate to cultures of lymphocytes.

Styrene-7,8-oxide did not induce morphological transformation of C3H/10T½Cl8 cells but enhanced the transforming activity of 3-methylcholanthrene in a two-stage transformation assay.

Responses to styrene-7,8-oxide *in vivo* are more variable. [The Working Group noted that the purity of the test compound was frequently stated in the publications but that the styrene-7,8-oxide used was probably a mixture of optical isomers, except when the R and S enantiomers were specified. Exposure was usually by inhalation or intraperitoneal injection (see Table 1).] DNA strand breaks were induced in a single study. Sister chromatid exchange was induced in mouse bone-marrow cells in one study with the S enantiomer of styrene-7,8-oxide but not with the R enantiomer. In another study, small increases in sister chromatid exchange frequencies were seen in liver cells and alveolar macrophages but not in bone-marrow cells in mice. A negative response was also obtained in bone-marrow cells of Chinese hamsters. Micronuclei were not induced by styrene-7,8-oxide in mouse or Chinese hamster bone marrow. Chromosomal aberrations were induced in mouse bone-marrow cells in one study with the S enantiomer of styrene-7,8-oxide but not with the R enantiomer. Conflicting results were obtained in two other studies with mice, since a significant increase in the frequency of bone-marrow cell chromosomal aberrations was

Table 1. Genetic and related effects of styrene-7,8-oxide

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, *Salmonella typhimurium umu*, SOS induction	+	0	0.0700	Nakamura *et al.* (1987)
ECB, *Escherichia coli* PQ37, SOS induction	+	0	100.0000	Głosnicka & Dziadziuszko (1986)
ECB, *Escherichia coli* PQ37, SOS induction	-	-	12000.0000	Brams *et al.* (1987)
ECB, *Escherichia coli* PQ37, SOS induction	+	0	36.0000	von der Hude *et al.* (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation (spot test)	+	0	200.0000	Milvy & Garro (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.6000	Vainio *et al.* (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	60.0000	de Meester *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	146.0000	Sugiura *et al.* (1978a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	250.0000	Wade *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	250.0000	Watabe *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	600.0000	Watabe *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	120.0000	Busk (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	125.0000	El-Tantawy & Hammock (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	240.0000	Yoshikawa *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.0000	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	768.0000	de Meester *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	144.0000	Sugiura & Goto (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	120.0000	Turchi *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	48.0000	Pagano *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	60.0000	Glatt *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	300.0000	Brams *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+[c]	+	500.0000	Hughes *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	0.0000	Claxton *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	60.0000	Einistö *et al.* (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	120.0000	Sinsheimer *et al.* (1993)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	+	768.0000	de Meester *et al.* (1981)
SA4, *Salmonella typhimurium* TA104, reverse mutation	+	0	120.0000	Einistö *et al.* (1993)
SA5, *Salmonella typhimurium* TA1535, reverse mutation (spot test)	+	0	5000.0000	Milvy & Garro (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.6000	Vainio *et al.* (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	24.0000	de Meester *et al.* (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	125.0000	Stoltz & Withey (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	60.0000	Loprieno *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	0	250.0000	Wade *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	50.0000	Watabe *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	60.0000	Busk (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	60.0000	El-Tantawy & Hammock (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.0000	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	768.0000	de Meester *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (spot test)	–	0	5000.0000	Milvy & Garro (1976)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	600.0000	Vainio *et al.* (1976)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	6000.0000	de Meester *et al.* (1977)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	0.0000	Wade *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	(+)	0	250.0000	Watabe *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	500.0000	El-Tantawy & Hammock (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.0000	De Flora (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1150.0000	de Meester *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation (spot test)	–	0	5000.0000	Milvy & Garro (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	+	6.0000	Vainio *et al.* (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	6000.0000	de Meester *et al.* (1977)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	0	250.0000	Watabe *et al.* (1978)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	0.0000	De Flora (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1150.0000	de Meester *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation (spot test)	–	0	5000.0000	Milvy & Garro (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	600.0000	Vainio *et al.* (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	6000.0000	de Meester *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	0.0000	Wade *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	250.0000	Watabe *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250.0000	Ueno *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	500.0000	El-Tantawy & Hammock (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	0.0000	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1150.0000	de Meester *et al.* (1981)
SA5, *Salmonella typhimurium* TA97, reverse mutation	+	0	300.0000	Brams *et al.* (1987)
SA5, *Salmonella typhimurium* TA4001, reverse mutation	+	0	240.0000	Einistö *et al.* (1993)
SA5, *Salmonella typhimurium* TA4006, reverse mutation	(+)	0	960.0000	Einistö *et al.* (1993)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	0	720.0000	Sugiura *et al.* (1978b)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	0	480.0000	Sugiura & Goto (1981)
KPF, *Klebsiella pneumoniae*, forward mutation	+	0	120.0000	Voogd *et al.* (1981)
SCG, *Saccharomyces cerevisiae*, gene conversion	+	0	1200.0000	Loprieno *et al.* (1976)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	0	600.0000	Loprieno *et al.* (1976)
ACC, *Allium cepa*, chromosomal aberrations and micronuclei	+	0	500.0000	Linnainmaa *et al.* (1978a,b)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+	0	1.0000 inhal.	Donner *et al.* (1979)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	0	36.0000	Sina *et al.* (1983)
DIA, DNA strand breaks, Pc12 cells *in vitro*	+	0	3.6000	Dypbukt *et al.* (1992)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	+	0	1020.0000	Loprieno *et al.* (1976)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	+	0	1020.0000	Bonatti *et al.* (1978)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	+	0	504.0000	Loprieno *et al.* (1978)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	+	–	240.0000	Beije & Jenssen (1982)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	(+)	0	100.0000	Nishi *et al.* (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	–	13.8000	Amacher & Turner (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	50.0000	de Raat (1978)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	0	20.0000	Nishi *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	0	15.0000	von der Hude *et al.* (1991)
MIA, Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	0	90.0000	Turchi *et al.* (1981)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	0	90.0000	Turchi *et al.* (1981)
TCM, Cell transformation, C3H10T1/2 mouse cells *in vitro*	–[d]	0	1.2000	Male *et al.* (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	8.4000	Norppa *et al.* (1981)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	1.0000	Pohlova *et al.* (1985)
MIH, Micronucleus formation, human cells *in vitro*	+	0	80.0000	Linainmaa *et al.* (1978a,b)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	60.0000	Fabry *et al.* (1978)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	80.0000	Linnainmaa *et al.* (1978a,b)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	24.0000	Norppa *et al.* (1981)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	3.0000	Pohlova *et al.* (1985)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* in mice	(+)		100×1, gavage	Loprieno *et al.* (1976)
HMM, Host-mediated assay, *Schizosaccharomyces pombe* in mice	(+)		100×1, gavage	Loprieno *et al.* (1976)
DVA, DNA strand breaks, mouse tissue *in vivo*	+		600×1, ip	Walles & Orsen (1983)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	–		86 inhal.×2	Norppa *et al.* (1979)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	–		500×1 ip	Norppa *et al.* (1979)
SVA, Sister chromatid exchange, mouse liver cells *in vivo*	(+)		72 inhal. 5 h×1	Conner *et al.* (1982)
SVA, Sister chromatid exchange, mouse alveolar macrophages *in vivo*	(+)		72 inhal. 5 h×1	Conner *et al.* (1982)
SVA, Sister chromatid exchange, mouse bone-marrow cells *in vivo*	–		72 inhal. 5 h×1	Conner *et al.* (1982)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+[e]		100×1 ip	Sinsheimer *et al.* (1993)
MVM, Micronucleus formation, BALB/c mouse bone-marrow cells *in vivo*	–		250×1 ip	Fabry *et al.* (1978)
MVC, Micronucleus formation, Chinese hamster bone-marrow cells *in vivo*	–		250×1 ip	Pentillä *et al.* (1980)
CBA, Chromosomal aberrations, BALB/c mouse bone-marrow cells *in vivo*	–		250×1 ip	Fabry *et al.* (1978)
CBA, Chromosomal aberrations, male CD-1 mouse bone-marrow cells *in vivo*	+		50×1, gavage	Loprieno *et al.* (1978)
CBA, Chromosomal aberrations, male Chinese hamster bone-marrow cells *in vivo*	–		86 inhal.×2	Norppa *et al.* (1979)
CBA, Chromosomal aberrations, Chinese hamster bone-marrow cells *in vivo*	–		500×1 ip	Norppa *et al.* (1979)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+[e]		100×1 ip	Sinsheimer *et al.* (1993)
DLM, Dominant lethal mutation, male mice *in vivo*	–		250×1 ip	Fabry *et al.* (1978)
BVD, Binding (covalent) to DNA, male CD rat stomach, liver *in vivo*			240×1 po	Cantoreggi & Lutz (1992)
BVD, Binding (covalent) to DNA, male B6C3F1 mouse liver *in vivo*	–		165×1 ip	Cantoreggi & Lutz (1992)
BVD, Binding (covalent) to DNA, male CD rat forestomach *in vivo*	(+)		1.3×1 po	Lutz *et al.* (1993)

[a] +, positive; (+), weakly positive; –, negative; 0, not tested; ?, inconclusive (variable responses in several experiments within an adequate study)
[b] In-vitro tests, μg/ml; in-vivo tests, mg/kg bw
[c] Incubated in Tedlar bags
[d] Positive in a two-stage assay
[e] S isomer only

reported in one study at a five-fold lower dose level than was used in another study in which no significant response occurred. No chromosomal aberrations were reported in a single study on Chinese hamster bone marrow.

No dominant lethal effect was observed in male mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Styrene-7,8-oxide is produced by cyclization of styrene chlorohydrin and by epoxidation of styrene with peroxyacetic acid. It is used mainly in the preparation of fragrances and as a reactive diluent in epoxy resin formulations. Few data are available on levels of occupational exposure to styrene-7,8-oxide. It has been detected in association with styrene, but at much lower levels, in industries where unsaturated polyester resins are used.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Styrene-7,8-oxide was tested for carcinogenicity in one experiment in mice and in two experiments in rats by oral gavage. It produced benign and malignant tumours of the forestomach in animals of each species and sex and induced hepatocellular tumours in male mice. It was also tested in one strain of rats by prenatal exposure followed by postnatal gastric intubation, producing benign and malignant tumours of the forestomach.

5.4 Other relevant data

Styrene-7,8-oxide is absorbed by rabbits and rats following its oral administration. In mice, the highest tissue concentrations are found in kidney, adipose tissue and blood. Styrene-7,8-oxide is hydrolysed rapidly in the acid environment of the stomach. Almost all of an administered dose of styrene-7,8-oxide is excreted in the urine of experimental animals. Styrene-7,8-oxide can be metabolized by epoxide hydrolase to the glycol or by glutathione *S*-transferase to glutathione conjugates. A small amount may be reduced to styrene. Styrene glycol is further metabolized to mandelic, phenyl glyoxylic and hippuric acids.

Styrene-7,8-oxide bound to histidine in haemoglobin and to cysteine in plasma proteins *in vitro*. Low levels of covalent binding to DNA were observed in the stomachs of orally dosed rats. In rat brain, it can decrease the activity of some neurotransmitters and monoamine oxidase, and it increases the availability of dopamine receptors. Glutathione *S*-transferase from human erythrocytes was inhibited by low concentrations of styrene-7,8-oxide.

No teratogenic effect was observed in rats or rabbits treated with doses of styrene-7,8-oxide up to the lethal level.

No data were available on the genetic and related effects of styrene-7,8-oxide in humans.

Both positive and negative results have been obtained with styrene-7,8-oxide for a variety of genetic end-points *in vivo*. Chromosomal aberrations and sister chromatid exchange were induced in mouse bone marrow only after treatment with the S enantiomer and not with the R enantiomer. DNA damage, mutations and chromosomal aberrations have been observed consistently in mammalian and nonmammalian systems *in vitro*.

5.5 Evaluation[1]

There *is inadequate evidence* in humans for the carcinogenicity of styrene-7,8-oxide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of styrene-7,8-oxide.

In making the overall evaluation, the Working Group took into consideration the following supporting evidence. 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*.

Overall evaluation

Styrene-7,8-oxide *is probably carcinogenic to humans* (*Group 2A*).

6. References

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Evidence for DNA and Protein Binding by Styrene and Styrene Oxide

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ABSTRACT: Styrene is metabolized to styrene oxide, a direct-acting mutagen and carcinogen. Styrene oxide reacts with DNA mainly at the *N-7* position in guanine, but also at other sites and with other bases. Substitution occurs at both the α- and β-positions of the styrene molecule. Experiments with radiolabeled styrene and styrene oxide demonstrate that both have a low level of DNA binding activity in experimental animals. ^{32}P-Postlabeling studies have demonstrated the potential of the technique to detect styrene-DNA adducts. Styrene oxide alkylates several nucleophilic sites in proteins, particularly cysteine sulfydryl, histidine imidazole, lysine amino, aspartic, and glutamic carboxylic groups, and the N-terminal position. In experimental animals, styrene oxide treatment results in cysteine adducts in hemoglobin and albumin, valine adducts in hemoglobin, and carboxylic acid adducts in hemoglobin. The extent of alkylation is low compared with that produced by ethylene oxide. The available evidence indicates, therefore, that styrene and styrene oxide have low DNA and protein binding activities *in vivo*. There is preliminary evidence for the presence of DNA adducts and for adducts in hemoglobin and albumin in blood cells of styrene-exposed workers. Nevertheless, the applicability and sensitivity of DNA and protein adduct detection methods for monitoring human exposure to styrene remain to be determined.

KEY WORDS: styrene, styrene oxide, DNA adducts, protein adducts, human biomonitoring, carcinogenicity, mutagenicity, genotoxicity.

I. INTRODUCTION

Styrene is an important industrial chemical that is widely used in the production of plastics, resins, and polymers. A major metabolite in mammals is styrene-7,8-oxide, which also is manufactured for use in epoxy resins and as a precursor for agrochemicals, cosmetics, and fibers.

Styrene oxide is a direct-acting mutagen and also is carcinogenic in experimental animals, inducing forestomach tumors in rats and mice.[1] Evidence for the carcinogenicity of styrene itself is more equivocal.[1] Both compounds show evidence of genotoxicity in a number of *in vitro* tests, activity with styrene usually requiring the presence of an exogenous metabolizing system.[2] The compounds also show activity in some *in vivo* genotoxicity assays, although species differences have been observed.[2,3]

These data raise the question of whether human exposure to styrene and styrene oxide poses a carcinogenic risk, and whether the carcinogenicity observed in experimental animals arises primarily through a genotoxic mechanism. Evidence of DNA and protein binding by carcinogenic agents in experimental animals generally is suggestive of a genotoxic mechanism of action.[4] In extrapolating from laboratory animals to man, DNA (or protein)-binding activity *in vivo* is widely regarded as indicating greater potential risk to humans than if the chemical is carcinogenic in animals by a nongenotoxic mechanism. In addition, the presence of protein- or DNA-bound styrene derivatives in human cells is a potentially useful monitor of occupational or environmental exposure.[5,6] This review considers the evidence for, and the nature of, DNA and protein binding by styrene and its oxide *in vivo* and *in vitro*.

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II. DNA ADDUCTS FORMED BY STYRENE OXIDE *IN VITRO*

Indirect evidence for DNA damage by styrene and its epoxide is provided by studies in which DNA strand breaks have been measured by alkaline elution. Exposure to styrene of rat hepatocytes, which have the ability to metabolize styrene to the epoxide, caused detectable strand breaks, while styrene oxide itself was active at a tenfold lower dose.[7]

Styrene oxide is a fairly reactive epoxide with a short half-life *in vivo*.[8] The rate at which it alkylates guanine is greatest for deoxyguanosine, intermediate for single-stranded DNA, and slowest for double-stranded DNA.[9]

There are four possible products from the reaction of styrene oxide with a given nucleophilic center in DNA. Ring-opening of the epoxide can generate a carbonium ion at either the 1- or 2-position of the 2-carbon sidechain. Reaction with nucleic acid leads to α- and β-carbon substitution, respectively. As the carbon atom at the 1-position has four different substituents, it is a chiral center, and thus a total of four diastereomers (two α, two β) are possible.

Reaction of styrene oxide with guanosine produced a variety of products that were purified and characterized by physicochemical methods.[10] Of the identified total of 90%, 57% were *N*-7 adducts, 28% were N^2 adducts, and about 15% were O^6 adducts. Approximately half were the result of β-substitution and half resulted from α-substitution of the styrene derivative (Figure 1).

Further work on the reaction of styrene oxide with deoxyribonucleosides and DNA identified similar adducts with dG and also adducts involving modification at the *N*-1 and N^6 positions of adenine, the N^4, *N*-3, and O^2 positions of cytosine, and the *N*-3 position of thymine.[11] Overall, the relative reactivity of the nucleosides was dG > dC > dA > T. The predominant product of the reaction with DNA was an *N*-7 guanine product, 41% of it involving the α-carbon as the reactive center and 59% involving the β-carbon. The relative amounts of α- and β-substitution were not obtained for the other adducts.

Of the four possible products of styrene oxide and guanosine at the O^6 position, the two β-adducts were found to be relatively stable, while the α-substituted adducts were readily hydrolyzed under mild acidic conditions.[12] Substitution at the β-position predominated over α-substitution by a factor of two.[12] In neutral or alkaline aqueous conditions, the α-diastereomers interconvert, as do the two β-substituted products.

When styrene oxide is reacted with thymidine monophosphate, phosphodiesters are formed.[13]

The advent of postlabeling methods for the detection of DNA damage has opened up the possibility of monitoring human exposure to carcinogens by seeking evidence for the presence of DNA adducts in human cells.[14] Before such methods can be applied routinely, however, it is necessary to determine the optimum conditions for the labeling and detection of given DNA adducts. The efficiency with which styrene-oxide-DNA adducts can be detected by ^{32}P-postlabeling analysis has been studied by Vodicka and Hemminki.[15] These investigators were unable to detect any ^{32}P-incorporation into the *N*-7-guanine alkylation products, while N^2-guanine adducts were labeled with 20% or lower efficiency and the O^6-guanine adducts with 5 to 10% efficiency. Thus, the most abundant DNA adducts are not detectable by ^{32}P-postlabeling, which tends to suggest that the ability of ^{32}P-postlabeling to detect styrene- or styrene oxide-DNA adducts formed at low levels will be limited. The inability to ^{32}P-label *N*-7 adducts efficiently has been observed with other alkylating species.[16,17]

Postlabeling of DNA reacted with tritium-labeled styrene oxide also was investigated by Cantoreggi et al.[18] Under standard labeling conditions, styrene oxide-DNA adducts were labeled with only poor efficiency (4 to 7% based on tritium incorporation). However, a modified protocol in which the ATP concentration was increased yielded a labeling efficiency of 35 to 55%. Although the four major adducts detected were not characterized, nor were their individual labeling efficiencies quantitated, this overall labeling efficiency suggests that the method can be applied to the detection of adducts formed *in vivo*.

Reaction of DNA with a tenfold excess of styrene oxide produced five adducts detected by ^{32}P-postlabeling.[19] These investigators observed

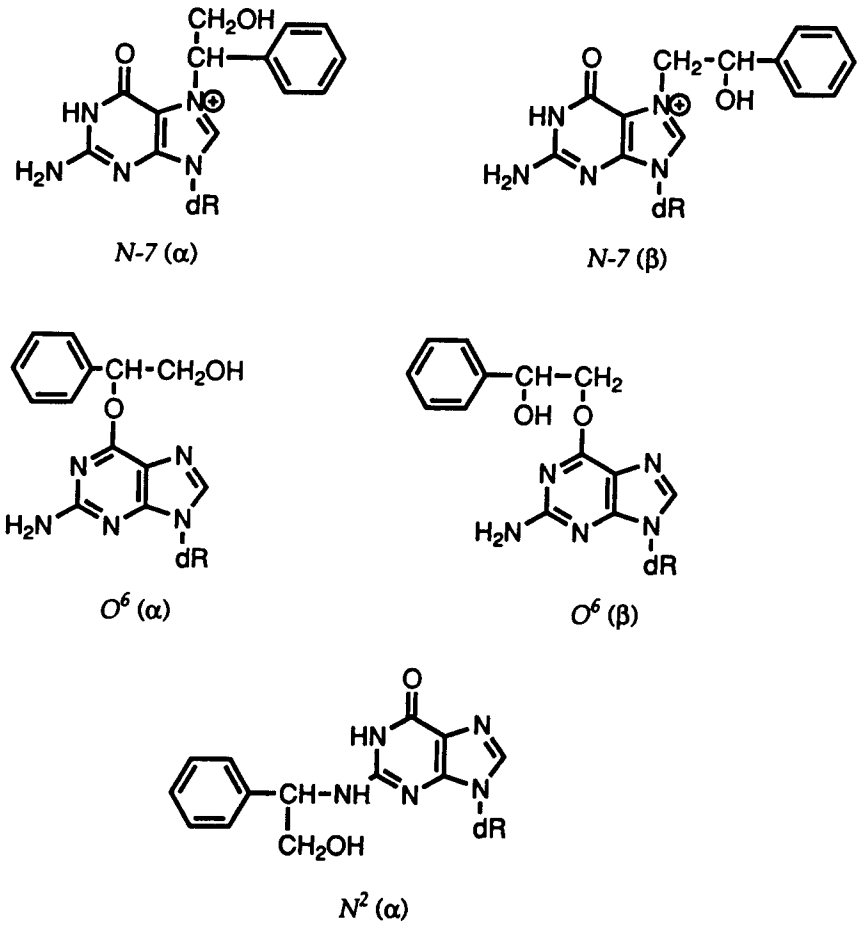
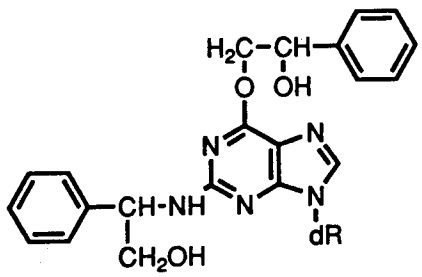


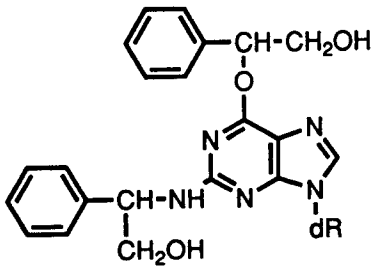
FIGURE 1. Identified structures of the major monoadducts formed from reaction of styrene oxide with DNA or deoxyribonucleosides. Styrene oxide also reacts with the *N*-1 and N^6 positions of deoxyadenosine, N^4, *N*-3, and O^2 positions of deoxycytidine, and *N*-3 position of thymidine. In each case, the α-carbon is chiral and two diastereomers are possible. dR, deoxyribose.

that five adducts also were formed by reaction with dGMP, two adducts with dAMP, and none with either dCMP or dTMP. The adducts detected with dAMP did not correspond to any of those detected in styrene oxide-modified DNA, in contrast to the results of Savela et al.,[11] who demonstrated adducts formed with both dA and dC and, to a minor extent, T.

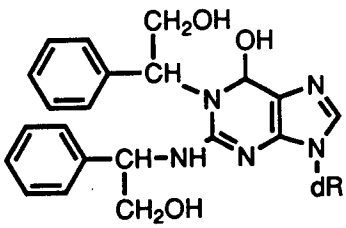
In two subsequent papers, Bodell and coworkers have characterized further the five styrene oxide-DNA adducts detected by ^{32}P-postlabeling. These were identified as including adducts involving substitution at the N^2 and O^6 positions of guanine.[20,21] Significantly, the major adducts included some that are described as being bis-styrene-oxide adducts; that is, they consist of a single nucleotide modified by two molecules of styrene oxide.[21] Subsequent characterization of these bis-adducts by mass spectrometry has identified the structure of three of them;[22] two of them are substituted at both the O^6 and N^2 positions of guanine, while a third involved *N*-1,N^2 bisubstitution of guanine (Figure 2). The formation of bis-adducts from the reaction of an electrophile with DNA appears to be a novel finding, but accounted for only about 2% of the total adducts in a DNA sample in which 1.7% of the DNA bases were modified by styrene oxide. The reac-



N^2,O^6 (α–β)



N^2,O^6 (α–α)



N-1,N^2 (α–α)

FIGURE 2. Structures of bis-styrene oxide-DNA adducts.

tion conditions for the formation of these adducts involved at least a tenfold excess of styrene oxide over nucleic acid; the yields of adducts at lower ratios were not reported. Thus, such species may be formed *in vitro* when the reactive compound is greatly in excess of the nucleic acid, but the significance for the formation of such species *in vivo*, where the amount of reactant relative to nucleic acid will be many orders of magnitude lower, is less clear.

As is the case with many adducts involving alkylation of the 7-position of guanine, those formed at this position by styrene oxide destabilize the glycosidic bond, leading to depurination. The half-life of the α-isomers in aqueous solution at neutral pH and 37°C is reported to be 440 min; that of the β-isomers is 250 min.[23] However, the rate of depurination of *N*-7-guanine adducts in double-stranded DNA is considerably slower ($t_{1/2} \approx 10$ d).[24]

Some differences in the relative amounts of different adducts formed with single- and double-stranded DNA have been noted.[25] With single-stranded DNA, the ratio of *N*-7, N^2, and O^6 alkylation products was 54:33:12, similar to the ratio obtained in reactions with guanosine,[10] while with double-stranded DNA the ratio was 74:23:3.7. Although these differences are interesting and indicate that steric factors influence the course of reaction, the magnitude of the disparity probably is not sufficiently large to have major biological consequences.

III. DNA ADDUCTS IN EXPERIMENTAL ANIMALS

The formation of DNA adducts by styrene and its epoxide *in vivo* has been studied using radiolabeled compounds. In mice following i.p. injection, ^{14}C-styrene binding to DNA in liver was reported.[26] A subsequent study[27] involved administration by inhalation. After mice and rats had been exposed to ^{3}H-styrene for up to 9 h, DNA was isolated from livers and lungs and purified to constant levels of radioactivity. Styrene was found to bind to DNA *in vivo,* but at a very low level; in the case of mouse liver, values were 20 to 50 times lower than those reported by Byfält-Nordqvist et al.,[26] the covalent binding index (CBI, [μmol adduct/mol DNA nucleotide]/[mmol chemical/kg body wt]) being between 0.05 and 0.09. In rat liver, no DNA adducts were detectable with a limit of detection of 0.1 CBI units.

In similar studies of adduct formation following administration of [7-^{3}H]styrene-7,8-oxide by oral gavage to rats and by i.p. injection to mice, much of the radioactivity found to be associated with isolated DNA resulted from isotope exchange, and the levels of radioactivity that could be attributed to the formation of DNA adducts again was found to be very low,[28,29] even in forestomach, in which styrene oxide induces tumors when administered at high dose. These authors concluded that DNA adducts formed at such low levels would be insufficient to account for the observed carcinogenic activity of styrene oxide by a purely genotoxic mechanism. Byfält-Nordqvist et al.[26] also tested [7-^{3}H]styrene-7,8-oxide, administered intraperitoneally, in mice and obtained a value for the extent of DNA binding similar to that obtained with styrene. However, this value is approximately 40-fold higher than the value obtained by Lutz and co-workers.[29]

The reason for these discrepancies in DNA binding levels is not immediately clear, even allowing for the fact that a variety of routes of administration (inhalation, ingestion, and i.p. injection) were used. Further, quantitation was by measuring radioactivity that coeluted with the α- and β-*N*-7-guanine adducts by one group of investigators,[26] and by measuring total radioactivity in DNA that had been purified to constant radioactive content by the other.[27-29] Even the expected slow loss of adducts by depurination[24] would not account for the observed differences. It is not immediately clear which values reflect more truthfully the real DNA binding levels, but in such circumstances (i.e., experiments with tritium-labeled compounds) it probably is wisest to accept the lower value as being the more reliable.

IV. DNA ADDUCTS IN STYRENE-EXPOSED WORKERS

Evidence has been presented for the formation of DNA single-strand breaks in peripheral lymphocytes in styrene-exposed workers.[30] Single-strand breaks can be induced in DNA during the repair of DNA damage. There was a correlation between the level of damage (i.e., breaks), the excretion of mandelic acid, and the concentration of styrene glycol in the blood, which suggests, along with the experimental induction of DNA strand breaks in animals treated with styrene and styrene oxide,[31] that the DNA damage was the result of exposure to styrene.

In two papers published in conference proceedings,[32,33] the analysis by ^{32}P-postlabeling of lymphocyte DNA from styrene-exposed workers has been reported. In the first report,[32] DNA from a single exposed worker was compared with a single "nonexposed" control. In the second report,[33] a single result again was presented, although it was not stated whether the results of both reports were obtained with the same individual. The authors claim to have observed evidence for the presence of styrene oxide-derived DNA adducts in the exposed worker(s).

The fact that adducts seen in the human DNA have chromatographic properties similar to those formed *in vitro* by styrene oxide should be treated with caution, considering that the tlc system used is a low-resolution procedure. Also, the conclusions are based on the results of the analysis of very few samples (one or two). Furthermore, the adducts in the exposed worker(s) appear to comigrate with adducts that Bodell and co-workers suggest are bis-styrene-oxide-dG adducts;[21,22] as stated above, even at very high levels of exposure, the formation of such adducts *in vivo* would

seem to be improbable, particularly in the apparent absence of any detectable monoadducts. Until such results are confirmed with a reasonable number of samples, these claims for evidence of the formation of styrene-related adducts in exposed workers must be regarded cautiously.

Recently, Hemminki and co-workers have reported the presence of adducts in the white blood cells of styrene-exposed workers.[34,35] Radioactivity that coeluted on tlc with O^6-dGMP adducts was present at levels five times higher in exposed workers than in controls. DNA from nonexposed individuals showed evidence of coeluting "background" radioactivity that was, nevertheless, unlikely to be due to styrene-DNA binding. As coelution in only one chromatographic system has been presented, it is not possible to rigorously exclude the possibility that the coeluting material is not due to styrene adducts, whether or not their presence was induced by chronic exposure to styrene. It also is somewhat anomalous that a minor styrene oxide-DNA adduct (≈4% of total binding *in vitro*) should apparently be detectable in the blood of styrene-exposed individuals when DNA binding occurs in the tissues of treated animals at only a very low level. Clearly, many unanswered questions arose from these preliminary reports; for example, the effect of longer-term chronic administration to animals on DNA adduct formation has not been investigated.

V. PROTEIN ADDUCTS FORMED BY STYRENE OXIDE *IN VITRO*

Styrene oxide alkylates a variety of nucleophilic sites within amino acids and proteins, including the cysteine sulphydryl group, histidine imidazole, aspartic and glutamic carboxylic groups, and the amino groups in lysine and at the N terminal of the protein chain. Initial *in vitro* experiments by Hemminki,[36] studying the reaction of the [^{14}C]-styrene oxide with polyamino acids, showed that polycysteine was most effective in forming adducts. Polyhistidine reacted at $^1/_{40}$th of the rate of polycysteine, and lesser amounts of adducts were detected in polylysine, polymethionine, polyarginine, and polyserine. Incubations of styrene oxide with the individual amino acids cysteine, histidine, lysine, and serine also demonstrated the superior reactivity of cysteine.[37] Two styrene oxide adducts with cysteine were isolated by high-performance liquid chromatography (HPLC) and shown by mass spectrometry (MS) to be α- and β-substituted isomers (ratio of about 2:1).

The only proteins that have been studied in detail for styrene oxide adduct formation are hemoglobin and albumin. In human blood that had been incubated with [^{14}C]-styrene oxide, the relative proportion of binding to hemoglobin, globin, and albumin has been reported[38] as 5.2:4.0:4.3. An independent estimate of globin binding by Sepai et al.[39] was 4.3% of the applied dose.

Mass spectrometric methods have been developed for determining a variety of styrene oxide-adducted amino acids isolated from these alkylated proteins. For example, the cysteine adduct may be determined by a procedure involving reaction with Raney nickel, which cleaves the thioether bond of the adduct, yielding a mixture of 1-phenylethanol and 2-phenylethanol.[40] These are derivatized with pentafluorobenzoylchloride and analyzed by gas chromatography (GC) with electron capture detection or by gas chromatography-mass spectrometry (GC-MS). The proportion of total styrene oxide adducts in human globin that is detected in this way is 5.8%. (This is in contrast to the earlier result obtained by Hemminki,[37] which suggested that the major hemoglobin adduct was at cysteine.) Linear dose-response curves were observed by Ting et al.[40] for the reaction of styrene oxide with the cysteine residues in both human and rat globin. Interestingly, rat globin contained 77 times more adduct than human globin at the same dose of styrene oxide. Rat hemoglobin contains a cysteine (β-Cys-125) not present in human hemoglobin that has been shown to have particularly high reactivity to other electrophilic agents.[41] The Raney nickel procedure for cysteine adducts has been applied more recently to the analysis of the reaction products of albumin with styrene oxide.[38] In human albumin, 76.4% of these products was accounted for by the cysteine product, that is, a 13-fold greater proportion of total blood modifi-

cation compared with the adduct on human globin cysteine. These data illustrate that, per milligram of protein, human albumin would be a more suitable target than globin for monitoring styrene oxide exposure. However, as there is about ninefold more hemoglobin than albumin per volume of human blood, the overall amount of each of these cysteine adducts is reasonably similar. The reactivity of rat albumin cysteine was similar to that of rat globin cysteine, which was thus greater than human albumin cysteine and much greater than human globin cysteine.

Alkylation of human globin also occurs at the amino group of the N-terminal valine, and analytical methods for this adduct have been developed by applying a modified Edman degradation procedure using the reagent pentafluorophenyl isothiocyanate.[26,42,43] The resulting pentafluorophenylthiohydantoin is determined by GC-MS using a stable isotope-labeled internal standard. *In vitro* experiments with [^{14}C]-styrene oxide showed that the extent of adduct formation at the N-terminal valine of human globin was 5.2% of total alkylation.[43] No studies have been made of the N-terminal alkylation of albumin.

Other nucleophilic sites where adducts of styrene oxide have been measured are the carboxylate residues of aspartic and glutamic acids. Phenylhydroxyethyl esters are formed that may be determined following their hydrolysis to styrene glycol (1-phenyl-1,2-ethane diol).[39,44,45] Quantitation may be achieved by GC-MS-selected ion recording using a stable isotope-labeled internal standard.[39] As a result of the instability of the phenylhydroxyethyl ester linkage to hydrolysis, pronase digestion of globin that had been reacted with styrene oxide released 2.7% of the total adducts as styrene glycol.[44,45] Mild alkaline hydrolysis of styrene oxide-adducted globin released 15% of total adducts as styrene glycol.[39] However, despite its relative instability, the carboxylic acid adduct has potential as an *in vivo* dose monitor for styrene oxide (see below).

Kaur et al.[46] have used sophisticated tandem mass spectrometric procedures to characterize chemically the nature and site of modification in hemoglobin alkylated *in vitro* by styrene oxide. Tryptic peptides were purified by HPLC and identified and sequenced mass spectrometrically. Histidine was shown to be a dominant site for alkylation (β-His-143, α-His-20), and evidence also was obtained for β-Cys-93 alkylation. Interestingly, it also was shown by Ferranti et al.[47] that the reaction of styrene oxide with angiotensin 1 was predominantly at two histidine residues and at a tyrosine residue. No quantitative methods have been developed for determining histidine adducts with styrene oxide, and the extent of histidine modification following treatment of globin with lower doses of styrene oxide is not known. However, if we accept the aforementioned figures of 5.8% alkylation at cysteine, 5.2% at valine, and 15% at carboxylic acids, then 74% of the adducts remain uncharacterized.

VI. PROTEIN ADDUCTS IN EXPERIMENTAL ANIMALS

A. Cysteine Adducts in Hemoglobin and Albumin

In rats that were administered 0.5, 1, 2, and 3 mmol of styrene per kilogram of body weight by i.p. injection, the cysteine adduct of styrene oxide was detected by the Raney nickel procedure,[40] and there was a linear increase in the amount of adduct produced with dose. Treatment with 1 mmol/kg styrene yielded 2.3 nmol of adduct per gram of globin. The detection limit of the method was 0.04 nmol of adduct per sample. Comparison of the data from the *in vivo* experiment with that from *in vitro* incubations of globin with styrene oxide indicated that only 0.015% of the styrene dose was available as styrene oxide in erythrocytes. However, such an estimate probably is not valid, as the *in vitro* experiment does not allow for metabolic detoxification of styrene oxide, as would occur *in vivo*. Cysteine alkylation also was determined following i.p. administration to rats of 0, 0.5, 1, and 3 mmol of styrene per kilogram of body weight and 0, 0.1, 0.3, and 1 mmol of styrene oxide per kilogram of body weight.[38] Dose-response curves for the alkylation of hemoglobin and albumin cysteine were linear and indicated that about 2% of the styrene dosage was available as styrene oxide in blood.

B. Valine Adducts in Hemoglobin

The first published report on the measurement of valine adducts in animals treated with styrene and styrene oxide is that of Byfält-Nordqvist et al.[26] These authors administered 7-[^{14}C]-styrene i.p. to mice at doses from 0.12 to 4.9 mmol/kg body weight, and 7-[^{3}H]-styrene oxide at doses from 0.037 to 1.1 mmol/kg body weight. With increasing doses of styrene oxide, a higher than proportional increase in the level of total alkylation of hemoglobin was seen at the highest doses, suggesting a saturation of a metabolic system. A similar response was seen with styrene-hemoglobin binding, (although total styrene-plasma protein binding showed a lower than proportional amount at high doses). In contrast to the results of Rappaport et al.[38] (see above), the degree of alkylation by styrene and styrene oxide was surprisingly similar. The valine adduct in hemoglobin was determined by the modified Edman procedure and constituted about 3% of the total alkylation.

Later studies were carried out by Tang et al.,[43] who monitored dose-response relationships for production of the valine-styrene oxide adduct in rats treated i.p. with 83 to 833 µmol of styrene oxide per kilogram of body weight. A linear response was observed, with adduct formation at the level of 0.19 nmol of adduct per gram of globin per millimole per kilogram of styrene oxide. Latriano et al.[48] exposed rats for 5 d, 6 h/d, to 1000 ppm of styrene and found a 25-fold increase in valine adducts compared with controls.

In mice treated i.p. with 0.4 to 2.1 mmol of styrene oxide per kilogram of body weight, Osterman-Golkar[49] showed a disproportionate increase in binding at the higher dose levels. A lesser but similar effect was seen in rats, which showed about threefold lower adduct levels at equivalent doses of styrene oxide. Mice dosed with styrene at the same doses showed that about 5% of styrene was available as the epoxide.

C. Carboxylic Acid Adducts in Hemoglobin

Rats dosed i.p. with 83 to 833 µmol of styrene oxide per kilogram of body weight were shown to contain hemoglobin carboxylic acid esters of styrene oxide[39] that increased in amount with dose (disproportionately at higher doses). The dose of 83 µmol of styrene oxide per kilogram of body weight yielded 16.7 pmol of adduct per gram of globin, and 833 µmol/kg yielded 724 pmol of adduct per gram of globin.

D. Summary

Low exposure levels of styrene/styrene oxide show linear dose-response relationships for protein adducts, although evidence seems to exist for metabolic saturation at high doses, which affects the linearity of the dose-response relationships. The "availability" of styrene oxide in blood following styrene treatment appears to be about 5% in mice and about 2% in rats. The *in vivo* experiments carried out to date have been with single, quite high doses of styrene/styrene oxide, and the extents of alkylation have been small in comparison, say, to ethylene oxide.[50]

VII. HEMOGLOBIN AND ALBUMIN ADDUCTS IN STYRENE-EXPOSED WORKERS

Very few studies have been reported on hemoglobin adducts in occupationally exposed populations. Brenner et al.[42] analyzed a variety of indicators of genotoxic exposure, including styrene oxide adducts with valine in hemoglobin in 14 styrene-exposed boatbuilders (exposure range, 0.6 to 44 ppm). One very much higher adduct level was observed (11159 in authors' units). The remainder of the exposed population (n = 13) had adduct levels in the range of 2.0 to 15.3, and the control group (n = 8) in the range of 0.002 to 24. No difference in the mean adduct levels was seen when the individual with the high adduct level was either excluded or included. However, this worker did excrete the highest levels of mandelic acid in the study and was in the "high" exposure category. The exposed and control groups were not perfectly matched; in particular, the exposed group had 43% cigarette smokers and the control group was all nonsmokers. An alternative non-

occupational source for the styrene oxide adduct cannot, therefore, be excluded.

Christakopoulos et al.[51] reported on the use of the Edman assay for N-terminal valine adducts for a group of reinforced plastics workers exposed to styrene. Seven of the 17 workers, with an estimated workplace concentration of styrene of 75 ppm, contained detectable adducts, with a mean amount of 28 pmol/g of globin. Three of the 11 controls had detectable levels of <13 pmol/g of globin.

The detection of occupational exposure to styrene through measurements of protein adducts has been demonstrated recently by Rappaport et al.[53] The population was a group of boat manufacturers (n = 48) exposed to styrene (range, 1 to 235 mg/m^3; mean, 64 mg/m^3). Styrene oxide-cysteine adducts were determined by the Raney nickel procedure.[38] In hemoglobin, the adduct levels ranged from 0.03 to 0.16 nmol/g protein (mean, 0.08 nmol/g) and did not correlate with exposure levels. However, in albumin, the levels were higher (0.119 to 3.8 nmol/g protein; mean, 1.8 nmol/g), reflecting the greater reactivity of albumin cysteine groups, and these levels correlated with the average exposure levels of the individuals. Background levels of the adducts were found in commercial albumin (0.98 ± 0.16 nmol/g protein) and hemoglobin (0.058 ± 0.004 nmol/g protein).

Other examples of human hemoglobin adduct measurement for styrene are not yet well reported. Sepai et al.[39] have carried out very limited assays in exposed workers (n = 6) but have not detected any carboxylic acid ester adducts.

VIII. CONCLUSIONS

Although there is evidence that styrene oxide is mutagenic in a number of test systems and reacts with nucleic acids *in vitro,* it appears to give rise to only a very low level of DNA binding, detected by the use of ^{3}H-labeled compound, *in vivo*. It is becoming increasingly apparent that there may be no absolute distinction between "genotoxic" and "nongenotoxic" carcinogens, with some compounds showing characteristics of both classes of agent.[4] The carcinogenicity of styrene and styrene oxide may be a consequence of a very weak genotoxic activity *in vivo* combined with a strong cell-proliferating activity.[52] Thus, it cannot be stated that the carcinogenicity of styrene oxide (and possibly of styrene itself) in experimental animals is the result of a purely, or even predominantly, genotoxic mechanism.

^{32}P-Postlabeling studies to date have failed to detect any radiolabeling of the major styrene oxide-DNA adducts formed at the *N*-7 position of guanine residues. Minor adducts are labeled with up to 20% efficiency, but it nevertheless is theoretically possible that the method is sufficiently sensitive to detect the formation of styrene oxide-DNA adducts *in vivo*. However, there have been no reports of studies on the detection by ^{32}P-postlabeling of styrene oxide-DNA adducts in experimental animals treated with either styrene or the epoxide.

In the absence of such studies, it is difficult to make predictions about the applicability of ^{32}P-postlabeling to biomonitoring studies of human exposure to styrene. As described in this review, evidence for the presence of styrene oxide-DNA adducts in human white blood cells, based on comigration of adduct spots with standards in a single chromatography system, has been presented. Further studies are clearly required to confirm this preliminary finding, the significance of which would also be considerably strengthened by the detection by ^{32}P-postlabeling of the same adducts in experimental animals treated with styrene or styrene oxide.

Styrene, via the production of styrene oxide, is not an effective alkylator of hemoglobin compared with other genotoxins that have been studied in this way. Osterman-Golkar et al.[50] quote the following adduct levels for human exposure (in picomoles per gram of globin per parts per million per hour): ethylene oxide, ~6; ethylene, ~0.2; butadiene, ~0.004; and styrene, ~0.001. This poor extent of alkylation is reflected in the high dose levels that experimenters have used in animal experiments in order to validate their monitoring methods. The paucity of published data on human biomonitoring and/or the difficulty of acquiring such data is therefore not surprising. The sensitivity of the adduct analyses needs to be considerably enhanced for their more widespread use for human exposure monitoring.

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