FINAL

Report on Carcinogens Background Document for

Styrene-7,8-oxide

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

Prepared for the:
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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).

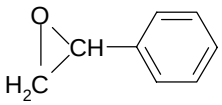

Figure 1-1. Structure of SO

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.

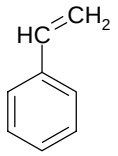

Figure 1-2. Structure of styrene

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.

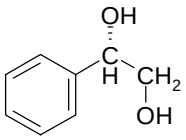

Figure 1-3. Structure of styrene glycol

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.

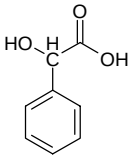

Figure 1-4. Structure of mandelic acid

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.

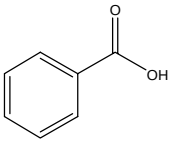

Figure 1-5. Structure of benzoic acid

Source: Chemfinder (1999)

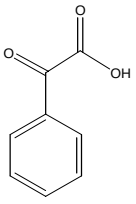

Figure 1-6. Structure of phenylglyoxylic acid

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.

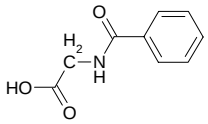

Figure 1-7. Structure of hippuric acid

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).

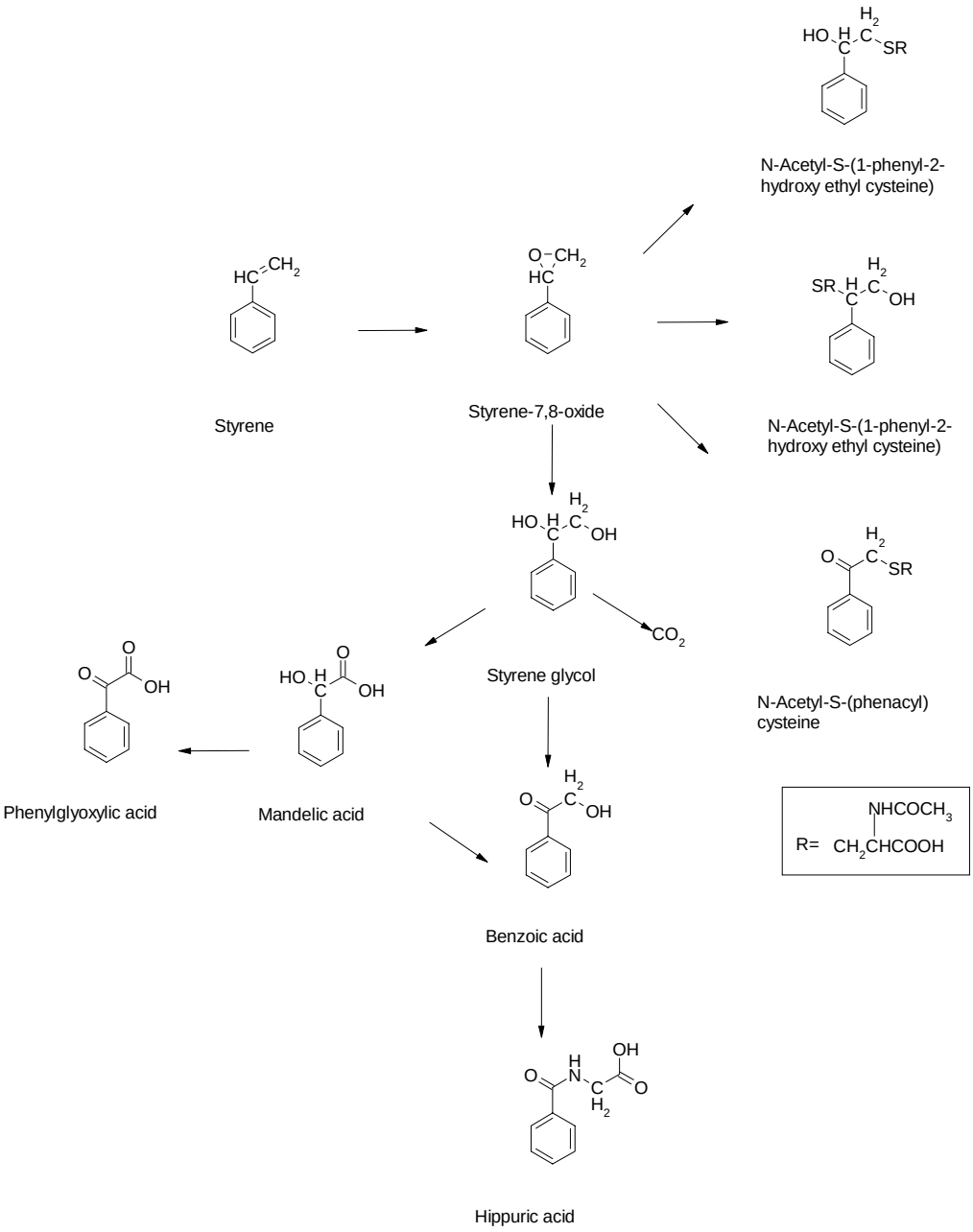

Figure 6-1. Metabolic pathways of SO

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).

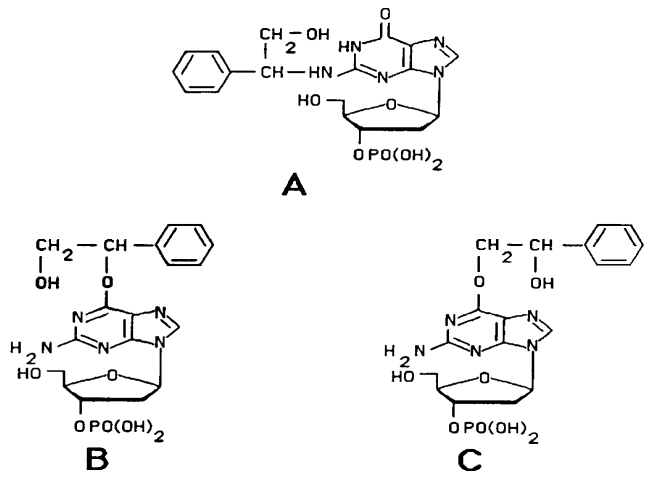

Figure 6-2. Structures of SO-DNA adducts detected by ^{32}P-postlabeling:

(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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