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.

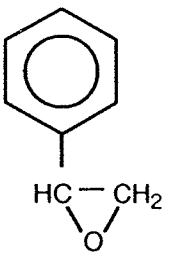
1. Exposure Data

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,β-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 °C
(*c*) *Freezing-point*: –36.7 °C

(*d*) *Density*: 1.050–1.054 at 20 °C/4 °C

(*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 °C); soluble in acetone, benzene, carbon tetrachloride, heptane and methanol

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

(*h*) *Stability*: Flash-point, 80–82 °C (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-oxide with

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[a]Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101.3 kPa)

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 B6C3F1 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 CD2F1 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 B6C3F1 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*-transferase. 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 B6C3F1 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), intermediate 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 β 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 (Vodička & 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 reported in one study at a five-fold lower dose level than was used in another study in which no significant response

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	Głosńicka & 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)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	0	300.0000	Brams *et al.* (1987)
SAS, *Salmonella typhimurium* TA4001, reverse mutation	+	0	240.0000	Einistö *et al.* (1993)
SAS, *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

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)*.

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[1]For definition of the italicized terms, see Preamble, pp. 27–30.

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