STYRENE

This substance was considered by previous Working Groups, in February 1978 (IARC, 1979), in March 1987 (IARC, 1987) and in February 1994 (IARC, 1994a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

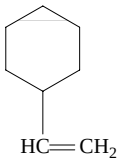
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 100-42-5
Replaced CAS Reg. No.: 79637-11-9
Chem. Abstr. Name: Ethenylbenzene
IUPAC Systematic Name: Styrene
Synonyms: Cinnamene; phenethylene; phenylethene; phenylethylene; styrol; styrole; styrolene; vinylbenzene; vinylbenzol

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_8H_8 Relative molecular mass: 104.15

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, viscous liquid with a pungent odour (WHO, 1983)
(*b*) *Boiling-point*: 145 ºC (Lide, 2001)
(*c*) *Melting-point*: –31 ºC (Lide, 2001)
(*d*) *Density*: d_4^{20} 0.9060 (Lide, 2001)

(*e*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991; Lide, 1996).

(*f*) *Solubility*: Insoluble in water; soluble in acetone, diethyl ether and ethanol (Lide, 2001). Very soluble in benzene and petroleum ether (WHO, 1983)

(*g*) *Volatility*: Vapour pressure, 867 Pa at 25 ºC; relative vapour density (air = 1), 3.6 (WHO, 1983)

(*h*) *Stability*: Lower flammable limit, 1.1% by volume in air (Quincy, 1991); flash point, 34 °C (National Institute for Occupational Safety and Health, 1983)

(*i*) *Reactivity*: Polymerizes easily at room temperature in the presence of oxygen and oxidizes on exposure to light and air (WHO, 1983)

(*j*) *Octanol-water partition coefficient* (*P*): log P = 2.95 (Hansch *et al*., 1995)

(*k*) *Conversion factor*: $mg/m^3 = 4.26 \times ppm$[1]

1.1.4 *Technical products and impurities*

Styrene is available as a commercial product with the following specifications: purity, 99.6–99.9% min.; ethylbenzene, 85 ppm max.; polymer content, 10 ppm max.; *para-tert-* butylcatechol (polymerization inhibitor), 10–15 ppm or 45–55 ppm; aldehydes (as benzaldehyde), 200 ppm max.; peroxides (as hydrogen peroxide), 0.0015 wt% or 100 ppm max.; benzene, 1 ppm max.; sulfur, 1 ppm typical; chlorides (as chlorine), 1 ppm typical (James & Castor, 1994; Chevron Phillips Chemical Co., 1996; Chen, 1997; Chevron Phillips Chemical Co., 2001).

Typical analysis of a commercial styrene product reported the following components: purity, 99.93%; benzene, < 1 ppm; toluene, < 1 ppm; ethylbenzene, 50 ppm; α-methylstyrene, 150 ppm; *meta-* + *para*-xylene, 240 ppm; *ortho*-xylene, 80 ppm; cumene, 70 ppm; *n*-propylbenzene, 40 ppm; *meta-* + *para*-ethyltoluene, 20 ppm; vinyltoluene, 10 ppm; phenylacetylene, 50 ppm; *meta-* + *para*-divinylbenzene, < 10 ppm; *ortho*-divinylbenzene, < 5 ppm; polymer, 1 ppm; *para-tert*-butylcatechol, 12 ppm; aldehydes (as benzaldehyde), 15 ppm; peroxides (as benzoyl peroxides), 5 ppm; chlorides (as chlorine), < 1 ppm; and sulfur, < 1 ppm (Chevron Phillips Chemical Co., 2001).

1.1.5 *Analysis*

(*a*) *Environmental monitoring*

Styrene in workplace air can be determined by capillary column gas chromatography (GC) with a flame ionization detector (FID). The sample is adsorbed on charcoal and desorbed with carbon disulfide. This method (NIOSH Method 1501) has an estimated limit of detection of 0.001–0.01 mg per sample (Eller, 1984).

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

EPA Method 8260B can be used to determine the concentration of various volatile organic compounds, including styrene, by GC–mass spectrometry (MS), in a variety of matrices, such as groundwater, aqueous sludges, waste solvents, oily wastes, tars, soils and sediments. Samples may be analysed using direct injection, purge-and-trap, closed-system vacuum distillation, static headspace (solid samples), or desorption from trapping media (air samples) (EPA Methods 5021, 5030, 5032, 5041); the practical quantification limits are 5 μg/L for groundwater samples, 5 μg/kg (wet weight) for low-level soil and sediment samples, 250 μg/L for water-miscible liquid waste samples, 625 μg/kg for high-level soil and sludge samples and 2500 μg/L for non-water-miscible waste samples (Environmental Protection Agency, 1996).

(*b*) *Biological monitoring*

Biological methods for monitoring exposure to styrene have been reviewed (Guillemin & Berode, 1988; Lauwerys & Hoet, 1993; Pekari *et al*., 1993; American Conference of Governmental Industrial Hygienists, 2001). Generally accepted biological markers of exposure are mandelic acid (2-hydroxy-2-phenylacetic acid) and phenylglyoxylic acid, the main metabolites of styrene (see Section 4.1.1(*c*)) in urine and styrene in blood. GC procedures have been described for the quantitative determination of urinary phenylglyoxylic and mandelic acids, which involve solvent extraction of the acids and their subsequent determination as derivatives by GC-FID on packed or capillary columns (Guillemin & Bauer, 1976; Flek & Šedivec, 1980; Bartolucci *et al*., 1986; Dills *et al*., 1991). High-performance liquid chromatography (HPLC) is widely used for determination of these metabolites. The acids, which may or may not be solvent-extracted, are separated on reverse-phase columns and quantified with an ultraviolet (UV) detector (Ogata & Sugihara, 1978; Ogata & Taguchi, 1987, 1988; Chua *et al*., 1993). Styrene has been determined in blood by GC with FID or mass selective detection either after solvent extraction (Karbowski & Braun, 1978) or by head-space techniques (Pezzagno *et al*., 1985; Bartolucci *et al*., 1986; Brugnone *et al*., 1993).

Measurement of adducts of styrene 7,8-oxide to the N-terminal valine in haemoglobin has been proposed for monitoring occupational exposure. After enrichment of adducted globin chains by ion-exchange chromatography, the samples are analysed by GC–MS after Edman degradation (Christakopoulos *et al*., 1993). Conjugation of styrene 7,8-oxide with glutathione, a minor metabolic pathway in humans, leads to specific mercapturic acid products that can be measured in the urine. Ghittori *et al.* (1997) measured these mercapturic acids and mandelic and phenylglyoxylic acids and styrene in the urine of 22 workers in a reinforced plastics plant, and assessed the correlation of urinary metabolites with time-weighted-average (TWA) styrene exposures measured by personal dosimetry. Correlation coefficients of individual or total mercapturic acids (as μg/g creatinine) with TWA styrene exposure ranged up to 0.56, compared with correlation coefficients of 0.86 and 0.82 for mandelic and phenylglyoxylic acids, respectively, with styrene exposure.

Methods of isotope-dilution GC–MS have been described for determination of styrene and styrene 7,8-oxide in blood. Styrene and styrene 7,8-oxide were measured directly in pentane extracts of blood from 35 reinforced plastics workers exposed to 4.7–97 ppm [20–414 mg/m^3] styrene. Positive ion chemical ionization allowed detection of styrene at concentrations greater than 2.5 μg/L blood and styrene 7,8-oxide at concentrations greater than 0.05 μg/L blood. An alternative method for measurement of styrene 7,8-oxide used a reaction with valine followed by derivatization with pentafluorophenyl isothiocyanate and analysis via negative ion chemical ionization GC-MS-MS (styrene 7,8-oxide detection limit, 0.025 μg/L blood). The detection limits for styrene 7,8-oxide by these two methods were 10–20-fold lower than those of the GC assays reported earlier, based upon either electron impact MS or FID (Tornero-Velez *et al.*, 2001).

Elia *et al*. (1980) found an excellent correlation (correlation coefficient, 0.96) between styrene exposure and urinary mandelic acid either alone or in combination with phenylglyoxylic acid. Ikeda *et al*. (1982) found a good correlation (correlation coefficient, 0.88) in 96 male workers in glass fibre-reinforced boat production plants between styrene concentration in air by personal sampling and the combined measurements of mandelic and phenylglyoxylic acid corrected for creatinine. Droz and Guillemin (1983) developed a biological model for the absorption, distribution and elimination of styrene. They examined its validity against data for 60 workers in 10 field studies in the polyester industry and found a good correlation.

Pezzagno *et al*. (1985) exposed 14 male and six female volunteers to styrene (273–1654 μmol/m^3 for 1–3 h) and found a correlation coefficient of 0.93 between exposure and levels of unchanged styrene in urine. Ong *et al*. (1994) studied 39 male workers exposed to concentrations of styrene less than 40 ppm [170 mg/m^3] (time-weighted average) and compared end-of-shift breath levels, blood concentrations of styrene, urinary styrene levels and urinary styrene metabolite levels with exposure levels; the best correlation was observed between blood styrene and styrene in air, while correlation was poor between styrene in urine and styrene in air. There were good correlations between styrene in air and mandelic acid and phenylglyoxylic acid in urine, but the best correlation was found between exposure and the sum of the two acids in urine corrected for creatinine.

Löf and Johanson (1993) exposed two volunteers to 26, 77, 201 and 386 ppm styrene (110, 328, 856 and 1672 mg/m^3) for 2 h during light (50 W) physical exercise. A nonlinear relationship between styrene in air and styrene in blood was observed which was attributed to metabolic saturation at exposure levels of 100–200 ppm.

1.2 Production

Styrene was first isolated in 1831 by distillation of storax, a natural balsam. Commercial production of styrene via dehydrogenation of ethylbenzene began in Germany in 1925 (Tossavainen, 1978; Lewis *et al*., 1983; National Institute for Occupational Safety and Health, 1983; Chen, 1997).

Styrene is produced mainly by catalytic dehydrogenation of high-purity ethylbenzene in the vapour phase. Typical catalysts are based on ferric oxide with the additives chromia (Cr_2O_3) (stabilizer) and potassium oxide (coke retardant) (Lewis *et al*., 1983). Fractionation of the product results in separation of high-purity styrene, unconverted ethylbenzene and minor reaction by-products such as toluene and benzene (WHO, 1983; James & Castor, 1994; Chen, 1997; Ring, 1999).

A smaller amount of styrene is produced as a co-product from a propylene oxide process. In this route, ethylbenzene is oxidized to its hydroperoxide and reacted with propylene to yield propylene oxide. The co-product methyl phenyl carbinol is then dehydrated to styrene (Mannsville Chemical Products Corp., 1987; Collins & Richey, 1992; James & Castor, 1994; Chen, 1997; Ring, 1999).

Data on the 1998 global production (and production capacity) and consumption of styrene by region are presented in Table 1.

Table 1. Worldwide supply and demand for styrene in 1998 (thousand tonnes)[a]

Region	Capacity	Production	Consumption
North America	6 763	6 095	5 241
South America	400	339	548
Western Europe	4 852	4 040	4 163
Eastern Europe	1 176	366	411
Middle East	555	518	275
Asia	7 294	6 503	7 155
Other[b]	112	84	119
Total	21 152	17 945	17 912

[a] From Ring (1999)
[b] Includes Africa and Oceania

Information available in 2001 indicated that styrene was produced by 21 companies in China, 10 in the USA, nine in Japan, six in Korea (Republic of), four each in Germany, Russia and the Ukraine, three each in Brazil, Canada, France, Mexico and the Netherlands, two each in Azerbaijan, Belgium, Singapore and the United Kingdom and one each in Argentina, Australia, Belarus, Bulgaria, Croatia, the Czech Republic, India, Iran, Poland, Saudi Arabia, Spain and Thailand (Chemical Information Services, 2001).

1.3 Use

Worldwide, styrene is one of the most important monomers for polymers and copolymers that are used in an increasingly wide range of applications. The major uses for styrene are in plastics, latex paints and coatings, synthetic rubbers, polyesters and styrene-alkyd coatings (Collins & Richey, 1992). The broad spectrum of uses of these

products includes construction, packaging, automotive and household goods (Mannsville Chemical Products Corp., 1987). Packaging is the single largest application for styrene-containing resins, particularly foams, used as fillers and cushioning. Construction applications include pipes, fittings, tanks, lighting fixtures and corrosion-resistant products. Household goods include synthetic marble, flooring, disposable tableware and moulded furnishings. Transport applications range from tyres to reinforced plastics and automobile body putty (Mannsville Chemical Products Corp., 1987).

Most styrene is converted to polystyrene resins, which are readily moulded and are compatible with a range of colourants, modifiers and fillers. These are used extensively in the fabrication of plastic packaging, disposable beverage tumblers, toys and other moulded goods. Expandable polystyrene beads are used for disposable cups, containers and packaging as well as for insulation. Copolymers and adducts are the second largest family of styrene derivatives. Acrylonitrile–butadiene–styrene (ABS) and styrene–acrylonitrile (SAN) resins have a variety of applications, including in appliance, automotive, construction, pipes and electronics mouldings (Mannsville Chemical Products Corp., 1987).

A variety of special resins have the styrene functionality. Styrene–butadiene rubber (SBR), used for tyres and other elastomer applications, is the largest volume synthetic rubber produced in the USA. Styrene–butadiene latex is used for carpet backing and paper processing. Styrene is the essential co-reactant and solvent in unsaturated polyesters used in reinforced plastic fabrications, including boats, corrosion-resistant tanks and pipes and automobile body parts (Mannsville Chemical Products Corp., 1987). Typical use patterns for styrene worldwide in 1998 and in Canada, Japan, Mexico, the USA and western Europe for selected years are presented in Tables 2 and 3.

Table 2. Use patterns for styrene worldwide in 1998 (thousand tonnes)[a]

Use	North America	Western Europe	Asia	Other	Total
Polystyrene	3 203	2 649	4 409	978	11 239
Unsaturated polyester resins	323	220	161	45	749
ABS/SAN resins	396	433	1 462	43	2 334
Styrene–butadiene copolymer latexes	410	383	279	44	1 116
SBR and latexes	263	123	270	185	841
Other	619	355	574	85	1 633
Total	5 214	4 163	7 155	1 380	17 912

ABS, acrylonitrile–butadiene–styrene; SAN, styrene–acrylonitrile; SBR, styrene–butadiene rubber
[a] From Ring (1999)

Table 3. Use patterns for styrene by country/region (thousand tonnes)[a]

Use	1985	1990	1994	1998
Canada				
Polystyrene	145	183	160	192
Unsaturated polyester resins	14	14	23	24
ABS resins	21	20	11	14
Styrene–butadiene copolymer latexes	25	26	23	11
SBR and latex	14	6	1	1
SAN resins	2	2	–	–
Other	6	7	20	20
Total	227	258	238	262
Japan				
Polystyrene	1 032	1 416	1 388	1 295
Unsaturated polyester resins	72	137	113	114
ABS/SAN resins	312	373	389	372
Styrene–butadiene copolymer latexes	87	129	146	158
SBR and latexes	93	104	86	98
Other	33	130	39	100
Total	1 629	2 289	2 161	2 137
Mexico				
Polystyrene	96	126	151	248
Unsaturated polyester resins	5	5	6	7
ABS resins	4	7	11	12
SBR and latex	29	24	22	28
SAN resins	1	1	1	3
Other	10	5	3	6
Total	145	168	194	304
USA				
Polystyrene	1 844	2 271	2 657	2 876
Unsaturated polyester resins	205	205	246	284
ABS resins	252	284	411	321
Styrene–butadiene copolymer latexes	363	455	562	642
SBR and latex	133	160	192	208
SAN resins	28	43	46	36
Other	123	122	130	308
Total	2 948	3 540	4 244	4 675
Western Europe				
Polystyrene	1 970	2 518	2 513	2 649
Unsaturated polyester resins	136	224	213	220
ABS/SAN resins	301	386	411	433
Styrene–butadiene copolymer latexes	182	286	361	383
SBR and latex	177	158	143	123
Other	248	278	334	355
Total	3 014	3 850	3 975	4 163

ABS, acrylonitrile–butadiene–styrene; SAN, styrene–acrylonitrile; SBR, styrene–butadiene rubber
[a] From Ring (1999); totals may not add due to independent rounding.

1.4 Occurrence

A comprehensive review on styrene exposure and health has appeared recently (Cohen *et al.*, 2002).

1.4.1 *Natural occurrence*

Styrene has been identified in trace amounts in the gummy exudate (Storax balsam) from the damaged trunks of certain trees, probably resulting from the natural degradation of the cinnamic acid derivatives that occur in large quantities in these exudates (Furia & Bellanca, 1971; Tossavainen, 1978; Duke, 1985). Styrene has been found at very low levels in many agricultural products and foods, but it is not clear whether this styrene results from natural processes within the plant (see Section 1.4.3 (*c*)).

1.4.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 1 112 000 employees in the USA were potentially exposed to styrene at work (National Institute for Occupational Safety and Health, 1993). The estimate was based on a survey of companies in the USA and did not involve actual measurements of exposure.

Workers may be exposed in a number of industries and operations, including styrene production, production of polystyrene and other styrene-containing polymer resins, plastics and rubber products fabrication, fabrication of reinforced-polyester plastics composites and use of products containing styrene, such as floor waxes and polishes, paints, adhesives, putty, metal cleaners, autobody fillers and varnishes (National Institute for Occupational Safety and Health, 1983).

(*a*) *Production of styrene and polystyrene*

Average exposure to styrene in styrene production and polymerization factories has been reported rarely to exceed 20 ppm [85 mg/m^3] and is usually due to occasional bursts and leakages of reactors, tubing and other equipment (Tossavainen, 1978). Surveys conducted in plants in the USA engaged in the development or manufacture of styrene-based products between 1962 and 1976 showed that the average exposure of employees in all jobs was below 10 ppm [43 mg/m^3]. Peak concentrations of up to 50 ppm [213 mg/m^3] were measured during the drumming of styrene. Batch polymerization of styrene in 1942 produced concentrations up to 88 ppm [375 mg/m^3] during filling operations; subsequent continuous polymerization processes generally resulted in exposure levels of 1 ppm [4.3 mg/m^3] or below (Ott *et al.*, 1980). In a plant in the USA where styrene was produced and polymerized, the highest levels of styrene were found in polymerization, manufacturing and purification areas (mean, 8–35 ppm [34–149 mg/m^3]), while levels of less than 5 ppm [21 mg/m^3] occurred in maintenance, laboratory and packaging operations. Urinary mandelic acid and blood styrene were undetectable in

most samples taken from these workers at the end of a shift: < 10 mg/g creatinine for mandelic acid (5 ng/mL) and < 2 ng/mL for styrene in blood. The maximal concentrations were 140 mg/g creatinine for mandelic acid and 90 ng/mL for styrene in blood (Wolff *et al*., 1978). In a German styrene production, polymerization and processing plant, samples taken in 1975–76 in various areas of the plant contained none (< 0.01 ppm [0.04 mg/m^3]) to 6.8 ppm [29 mg/m^3], most values being below 1 ppm [4.3 mg/m^3]. In a part of the plant where polystyrene was manufactured, area samples in 1975 contained from none (< 0.01 ppm [0.04 mg/m^3]) to 47 ppm [200 mg/m^3], most values being below 1 ppm [4.3 mg/m^3]. Of 67 employees engaged in either area of the plant, six had urinary concentrations of mandelic acid above 50 mg/L (Thiess & Friedheim, 1978).

Other substances that may be found in workplace air during the manufacture of styrene and polystyrene include benzene, toluene, ethylbenzene, other alkylbenzene compounds and ethylene (Ott *et al*., 1980; Lewis *et al*., 1983; National Institute for Occupational Safety and Health, 1983). Exposure to benzene was previously a primary concern in these processes. In the plant in the USA described above, the TWA concentration of benzene in styrene monomer manufacture was 0.3–14.7 ppm [1–47 mg/m^3] between 1953 and 1972. Samples taken in 1942 during a washing operation in the polymerization plant contained up to 63 ppm [202 mg/m^3] benzene (Ott *et al*., 1980).

(*b*) *Production of styrene–butadiene rubber (SBR) and other styrene-based polymers*

Concentrations of styrene in area samples and breathing-zone air measured in 1965 in various plants of a styrene–butadiene latex manufacturing company in the USA (see above) were 4–22 ppm [17–94 mg/m^3]. The initial stages of the process, including loading, operating and cleaning of polymerization reactors, involved the highest exposure, and operators in these job categories were exposed to concentrations ranging from 3.6 to 7.3 ppm [15–31 mg/m^3] in 1973 (Ott *et al*., 1980).

In two adjacent SBR production plants in the USA, the TWA concentrations of styrene were 0.94 and 1.99 ppm [4 and 8.5 mg/m^3], with an overall range of 0.03–12.3 ppm [0.13–52.4 mg/m^3] (Meinhardt *et al*., 1982). The mean concentrations in 159 personal air samples taken in 1979 in various departments at another SBR production plant in the USA were usually below 1 ppm [4.3 mg/m^3], except for factory service and tank farm workers, for whom the means were 1.69 and 13.7 ppm [7.2 and 58.2 mg/m^3], respectively (Checkoway & Williams, 1982). Company data provided by five of eight SBR plants in the USA for the period 1978–83 gave an average styrene level in 3649 samples from all plants of 3.53 ppm [15 mg/m^3], with a standard deviation of 14.3 ppm [61 mg/m^3] (Matanoski *et al*., 1993). A study by Macaluso *et al*. (1996) of the same facilities as the Matanoski study used industrial hygiene data together with a series of air dispersion models to estimate how TWA styrene exposure levels in the styrene–butadiene resin industry may have changed since the 1940s. Their calculations suggest that TWA exposures declined from an average of 1.8 ppm [7.7 mg/m^3] during the 1940s to 0.1 ppm [0.4 mg/m^3] in the 1990s.

In a plant in the USA where acrylic ester–styrene copolymers [wrongly called polystyrene by the authors] were produced, concentrations in the breathing zone in 50 samples ranged from none detected (less than 1 ppb [4.3 μg/m^3]) to 19.8 ppm [84 mg/m^3], with an average of about 0.6 ppm [2.5 mg/m^3]; the highest concentrations occurred during styrene unloading operations (Samimi & Falbo, 1982).

The numerous other substances to which workers may be exposed in these processes include 1,3-butadiene, acrylonitrile, acrylates, acrylic acid, α-methylstyrene (*meta*-vinyltoluene), 4-vinylcyclohexene, toluene, benzene, ammonia, formaldehyde, colourants and a variety of solvents (Ott *et al.*, 1980; Samimi & Falbo, 1982; National Institute for Occupational Safety and Health, 1983). Accelerators are chemical compounds that increase the rate of cure and improve the physical properties of natural and synthetic rubbers, including SBR. Thiuram sulfides and salts of dialkyldithiocarbamic acids, including dimethyldithiocarbamate, are used as vulcanization accelerators. Sodium and potassium dimethyldithiocarbamates are used as modifiers in emulsion polymerization (Schubart, 1987; Lattime, 1997).

(*c*) *Processing of styrene-based polymers*

Styrene was measured as a thermal degradation product in the air of a Finnish factory during the processing of polystyrene, impact polystyrene and acrylonitrile–butadiene–styrene (ABS) resins. The mean concentrations (6 h) were 0.4, 0.1 and 0.06 mg/m^3, respectively (Pfäffli, 1982). Personal 8-h samples taken in 1978, 1979 and 1980 in companies in the USA where polystyrene and ABS moulding was performed contained [17–285 mg/m^3] (Burroughs, 1979); 1.4–3.2 mg/m^3 (Belanger & Elesh, 1980) and < 0.01 mg/m^3 (below the limit of detection) (Ruhe & Jannerfeldt, 1980).

Styrene is one of the volatile organic compounds released during extrusion and vulcanization of SBR. Rappaport and Fraser (1977) reported styrene concentrations of 61–146 ppb [0.3–0.6 mg/m^3] in the curing area of the press room of a company manufacturing passenger-car tyres. Area samples taken in the vulcanization and extrusion areas of shoe-sole, tyre retreading and electrical cable insulation plants contained styrene at concentrations of 2–500 μg/m^3 (vulcanization) and 0–20 μg/m^3 (extrusion) (Cocheo *et al*., 1983). A more complete description of the work environment encountered in the rubber products manufacturing industry may be found in a previous monograph (IARC, 1982).

(*d*) *Manufacture of glass fibre-reinforced polyester products*

Occupational exposure to styrene is most extensive, with respect to number of workers and levels of exposure, in the fabrication of objects from glass fibre-reinforced polyester composite plastics, such as boats, tanks, wall panels, bath and shower units and automotive parts (National Institute for Occupational Safety and Health, 1983). Styrene serves as a solvent and a reactant for the unsaturated polyester resin, in which it constitutes about 40% by weight. In the open mould process, a releasing agent is usually applied to the mould, a first coat containing pigments (gel coat) is applied, then

successive layers of chopped and/or woven fibre glass are deposited manually or with a chopper gun at the same time as the resin is sprayed or brushed on, and then the surface is rolled. During lamination and curing, about 10% of the styrene may evaporate into the workplace air (National Institute for Occupational Safety and Health, 1983; Crandall & Hartle, 1985). Exposure to styrene in this industry has been extensively documented and summarized in several reports (National Institute for Occupational Safety and Health, 1983; WHO, 1983; Pfäffli & Säämänen, 1993). Table 4 lists levels of occupational exposure to styrene (personal breathing zone samples) reported in various countries in the larger studies.

Among the biological monitoring methods available (Section 1.1.5(*b*)), measurements of mandelic acid and phenylglyoxylic acid (see Section 4.1.1(*c*)) in urine are the most commonly used biological indices of exposure to styrene. Table 5 gives the concentrations of the classical biological indicators of exposure from various studies. Symanski *et al*. (2001) examined the variation in urinary levels of mandelic acid and phenylglyoxylic acid among workers exposed to styrene in the reinforced plastics industry. Levels of phenylglyoxylic acid varied less than those of mandelic acid, as did metabolite levels expressed in terms of urinary creatinine concentration. Urinary metabolite levels were highest for laminators and for samples collected at the end of the working week.

Several factors influence the level of styrene in air. The manufacture of objects with large surface area, such as boats, truck parts, baths and showers, by the open-mould process results in the highest exposure. Data from 28 plants producing reinforced plastics products in the USA showed that the average exposure to styrene in open-mould processes was two to three times higher than that in press-mould processes: 24–82 ppm [102–350 mg/m^3] versus 11–26 ppm [47–111 mg/m^3] (Lemasters *et al*., 1985). In a detailed survey of 12 plants making fibreglass in Washington State, USA, 40% of 8-h samples contained more than 100 ppm [430 mg/m^3]. Chopper gun operators had the highest exposure, followed by laminators and gel-coat applicators; boat-building involved higher exposures than any other sector. For 11 plants, a relationship was seen between level of exposure and the quantity of resin consumed per month per exposed employee (Schumacher *et al*., 1981). Similar results were reported by Sullivan and Sullivan (1986) in their survey of 10 plants in Ontario, Canada, who also noted that although dilution ventilation and often auxiliary fans were used in almost all plants, there was little use of local exhaust ventilation. This was also the case for boat construction in the USA. Gel coaters have lower exposure because they generally work in ventilated booths (Crandall & Hartle, 1985). The presence of flexible exhaust ventilation hoses was reported to reduce styrene concentrations by a factor of two at a boat construction company in Japan (Ikeda *et al*., 1982). So-called ‘low-styrene emission resins’ are in theory promising for reducing exposure, but their potential to do so in the workplace has not been sufficiently validated and they are not widely used (A.D. Little, Inc., 1981; Sullivan & Sullivan, 1986; Säämänen *et al*., 1993).

An extensive data source on exposures to styrene in the styrene composites manufacturing industry is the report of a study conducted in 1986 by Cal/OSHA (1986). This

Table 4. Occupational exposure to styrene in the glass fibre-reinforced plastics industry in various countries

Country and year of survey	No. of plants	Job/task	Duration of samples	No. of samples	Air concentration in personal breathing zone (mg/m^3)		Reference
					Mean	Range	
Canada (Ontario)	10	All jobs	25 min	126	< 4.3–716[a]	< 4.3–1393	Sullivan &
1981		Boat laminating		59	430 GM	8.1 GSD	Sullivan (1986)
		Non-boat laminating		23	124 GM	29.4 GSD	
		Chopper gun use		8	554 GM	7.7 GSD	
		Gel-coat spraying		6	298 GM	7.7 GSD	
		Filament winding		3	533 GM	6.0 GSD	
Canada (Québec)	3	Chopper gun use	8 h	7	564	307–938	Truchon *et al.*
NR		Painting (gel coat)		9	517	280–843	(1992)
		Laminating (rollers)		18	502	292–865	
		Foreman		8	97	18–279	
		Cutter		11	75	16–234	
		Warehouse work		19	35	9–187	
		Finishing		31	34	8–110	
		Mould repair		8	28	8–147	
Denmark							Jensen *et al.*
1955–70	30	NR	1–60 min	227	714	10–4700	(1990)
1971–80	97	NR		1117	274	4–1905	
1981–88	129	NR		1184	172	1–4020	
Italy	87	Hand laminating	Variable	1028	227		Galassi *et al.*
1978–90		Spraying laminating		166	134		(1993)
		Rolling		40	163		
		Semi-automatic process		71	85		
		Non-process work		159	71 (38 GM)	3.8 GSD	
Italy	10	NR	8 h	64	113.6 GM	8–770.4	Gobba *et al.*
NR							(1993)
Japan	5	Boat fabrication:	4 h				Ikeda *et al.*
NR		Hull lamination		25	507 GM	145–1091	(1982)
		Hull lamination with local exhaust ventilation		9	277 GM	196–383	
		Lamination of hold walls		25	537 GM	371–916	

Table 4 (contd)

Country and year of survey	No. of plants	Job/task	Duration of samples	No. of samples	Air concentration in personal breathing zone (mg/m^3)		Reference
					Mean	Range	
Switzerland NR	10	NA	Full shift	90	201	8–848	Guillemin *et al.* (1982)
Netherlands NR	4	Filament winding Spraying Hand laminating	4 h	18 62 180	[314 GM] [227 GM] [148 GM]	134–716 48–602 18–538	Geuskens *et al.* (1992)
USA NR	7	Boat fabrication Hull lamination Deck lamination Small parts lamination Gel coating	Full-shift	 168 114 70 45	 331 313 193 202	 7–780 52–682 34–554 23–439	Crandall & Hartle (1985)
Europe (5 countries)[b]		Lamination	Variable				Bellander *et al.* (1994)
< 1980		Boat fabrication		1703	332		
≥ 1980				2993	234		
< 1980		Containers		437	247		
≥ 1980				1098	187		
< 1980		Panels and construction		401	213		
≥ 1980				846	145		
< 1980		Small pieces		486	251		
≥ 1980				629	158		
≥ 1980		Hand lamination		3205	281		
≥ 1980		Spray lamination		414	132		
≥ 1980		Non-manual lamination		231	68		

Table 4 (contd)

Country and year of survey	No. of plants	Job/task	Duration of samples	No. of samples	Air concentration in personal breathing zone (mg/m^3)		Reference
					Mean	Range	
USA	30	Spray-up/lay-up	≥ 60 min; 8-h TWA computed	NR	256[c]	21–511	A.D. Little, Inc. (1981)
1967–78		Gel coating			192	43–256	
		Winding			170	64–362	
		Sheet-moulding compound production			170	43–341	
		Foaming			128	64–213	
		Mixing			107	9–341	
		Casting			85	21–192	
		Cut, press and weigh			64	21–341	
		Other jobs[d]			≤ 43	0–213	

GM, geometric mean; GSD, geometric standard deviation; NA, not applicable; NR, not reported; TWA, time-weighted average; concentrations originally reported in ppm were converted to mg/m^3 for this table

[a] Range of arithmetic means for different plants

[b] Italian plants reported by Galassi *et al*. (1993) and Finland, Norway, Sweden and the United Kingdom

[c] Typical level

[d] Includes general and non-production, finish and assembly, store and ship, office and other, injection molding, field service, preform production and pultrusion (a continuous process for producing composite materials of constant cross-sectional area)

Table 5. Biological monitoring of occupational exposure to styrene in the glass fibre-reinforced plastics industry

Country and year of survey	No. of plants	Job/task	No. of samples	Concentrations at end of shift						Reference
				Mandelic acid in urine (mg/g creatinine)		Phenylglyoxylic acid in urine (mg/g creatinine)		Styrene in blood (mg/L)		
				Mean	SD	Mean	SD	Mean	SD	
Canada (Quebec) NR	3	Chopper gun operation Painting (gel coat) Laminating (rolling) Foreman Cutting Warehouse worker Finishing Mould repair	7 9 18 8 11 19 31 8	[980] [750] [1690] [350] [320] [70] [110] [30]	[980] [310] [605] [470] [380] [70] [120] [50]					Truchon *et al*. (1992)
Germany NR	4	Laminating boats, pipes, or containers	36	210 (10–3640)[a]		190 (10–870)[a]		0.39 (0.04–4.82)[a]		Triebig *et al*. (1989)
Italy NR	4	Refrigerating containers Flooring tiles Fibre-glass canoes Fibre-glass tanks	6 6 5 3	493 428 270 323	434 248 54 129	121 72 62 132	96 22 24 41	0.32 0.42 0.52 NR	0.42 0.16 0.32	Bartolucci *et al*. (1986)
Italy 1978–90	118	Hand lamination Spray lamination Rolling Semi-automatic process operation Non-process work	2386 250 63 121 762	450 GM 211 GM 182 GM 154 GM 94 GM	2.75 GSD 3.3 GSD 3.08 GSD 259 GSD 3.27 GSD					Galassi *et al*. (1993)
Switzerland NR	10	NR	88	1004	1207	339	360			Guillemin *et al*. (1982)
United Kingdom 1979	1	Boat industry	27	[780]	555			[0.72]	[0.43]	Cherry *et al*. (1980)

SD, standard deviation; NR, not reported; GM, geometric mean; GSD, geometric standard deviation
[a] Median (range) in mg/L

study was an in-depth industrial hygiene survey of styrene and other workplace exposures. A total of 141 workplaces with 2600 workers were inspected, and in 50 of these workplaces, a total of 379 workers were monitored over a full work shift. Exposures were sampled by a charcoal tube method with personal sampling pumps, and in addition with passive organic vapour dosimeters, and by analysis of the urine of 327 workers (85% of the study population) for mandelic acid. The focus of the study was on large open-mould spray-up/lay-up operations. Styrene exposures at these processes ranged from 0.2 to 288 ppm [0.85–971 mg/m^3] TWA for eight hours; the 8-h TWA arithmetic mean and the median for these sample results were 43.0 ppm [183 mg/m^3] and 34.0 ppm [145 mg/m^3], respectively. In a comparison of worker exposure levels by industry, the Cal/OSHA study showed that the geometric mean exposure levels were highest in tub/shower manufacturing facilities (53.6 ppm [228 mg/m^3]), followed by camper manufacturing (41.0 ppm [175 mg/m^3]), boat manufacturing (29.1 ppm [124 mg/m^3]), spa manufacturing (25.8 ppm [110 mg/m^3]), miscellaneous manufacturing (22.0 ppm [94 mg/m^3]), and tank manufacturing facilities (12.7 ppm [54 mg/m^3]). Operations ranked according to percentage of styrene exposures above 100 ppm [430 mg/m^3] as an 8-h TWA were: tub/shower manufacturing (19%), spa manufacturing (11%), camper manufacturing (6%), miscellaneous plastics manufacturing (4%) and boat and tank manufacturing (none).

Styrene exposures were measured for 82 Dutch workers at four plants where unsaturated polyester resins were applied to moulds in the production of glass fibre-reinforced plastics (Geuskens *et al*., 1992). The study considered three resin application categories: filament winding techniques; hand laminating or spraying using more than 25 kg resin every 4 h; hand laminating using less than 25 kg resin per 4 h; workers who were in the production area but did not come into direct contact with styrene were the control group. The geometric mean 8-h TWA concentrations for the four plants ranged from 11 to 49 ppm [47–209 mg/m^3] for the workers who came into direct contact with styrene and from 5 to 16 ppm [21–68 mg/m^3] for those who were in the production area but had no contact with styrene.

Wong *et al*. (1994) followed 15 826 American workers in the reinforced plastics industry who had worked in areas with exposure to styrene for at least six months between 1948 and 1977. Using estimates of exposure based on current (around 1980) measurements and a historical assessment of work practices, plant design, process design and occupational hygiene factors, they divided their cohort into strata by cumulative styrene exposure. Approximately 23% of the workers had estimated cumulative exposures exceeding 100 ppm–years. The estimated average exposure among members of this group was 298 ppm–years.

The Norwegian National Institute of Occupational Health reviewed a collection of more than 7000 styrene concentration measurements taken in 234 reinforced-plastics production companies in Norway during the years 1972 through 1996 (Lenvik *et al*., 1999). Of these plants, 124 (accounting for 60.2% of the measurements) produced boats, 65 (accounting for 30.2%) produced small items, five (accounting for 3.4%) produced car body parts and another 40 (accounting for 6.2%) produced miscellaneous items.

Since 1990, the long-term average measurements (sampling time more than 1 h) fell below 20 ppm [85 mg/m^3] (except for a value slightly above 20 ppm in 1996). The analyses show a decrease in the median from 62 ppm [264 mg/m^3] in the 1970s to 7.1 ppm [30 mg/m^3] in the 1990s.

A comprehensive approach to biological monitoring of 44 workers occupationally exposed to styrene in a hand lamination plant in Europe was performed by considering several end-points, including styrene in workplace air, styrene in exhaled air, and styrene in blood. Other end-points included DNA strand breaks, chromosomal aberrations, immune parameters and genotyping of polymorphic genes (see Section 4.5). The set of workers consisted of four groups: the high-exposure group, consisting of hand lamination workers; the medium-exposure group, consisting mainly of sprayers; the low-exposure group, consisting of maintenance workers; and the control group, consisting of non-exposed clerks. The mean duration of exposure for the whole group was 13.0 years. The mean concentration of styrene in the workplace for the whole group was 101 mg/m^3 and the mean blood concentration of styrene was 601 μg/L. For the high-, medium- and low-exposure groups, mean air (and blood) concentrations were 199 mg/m^3 (2098 μg/L), 55 mg/m^3 (81 μg/L) and 27 mg/m^3 (85 μg/L) (Somorovská *et al*., 1999).

Measurement of biological indicators of exposure complements the picture based on air concentrations because biological levels incorporate the influence of other routes of absorption and of the use of personal protective equipment (see also Section 4.1). Despite early reports that percutaneous absorption of styrene was an important route of exposure, measurement of biological indicators of the exposure of workers who did and did not wear gloves and other forms of protective clothing indicated that absorption through the skin makes a negligible contribution to overall exposure in the manufacture of glass fibre-reinforced polyester products (Brooks *et al*., 1980; Bowman *et al*., 1990; Truchon *et al*., 1992). Wearing a respirator appropriate for organic vapours reduces exposure markedly, but not entirely (Brooks *et al*., 1980; Ikeda *et al*., 1982; Bowman *et al*., 1990; Truchon *et al*., 1992). Respirators are worn most often by gel-coat and chopper gun operators but not by laminators, who consider that they hinder their work (Truchon *et al*., 1992). Single-use dust respirators, which provide no protection against styrene vapours, were often the only type of protection worn (Schumacher *et al*., 1981; Sullivan & Sullivan, 1986).

Limasset *et al*. (1999) compared the level of styrene absorbed percutaneously with that absorbed by inhalation in a real situation in the glass fibre-reinforced polyester industry. The study protocol consisted of comparisons of the patterns of urinary excretion of styrene metabolites by four groups of workers, all of whom performed the same task at the same time in the same workshop but wore the following different protective equipment: total protection with an insulating suit and mask, respiratory equipment only, skin protection only, and no protection. The urinary excretion level of the group with total protection did not significantly differ from that of the group with respiratory protection only. Percutaneous absorption was not a particularly important pathway for styrene absorption during stratification work in the polyester industry.

Completely insulating personal protective equipment provided no greater level of protection than does a respirator at positive pressure alone.

The protection afforded by respirators to styrene-exposed workers has also been evaluated by measuring the reduction in urinary excretion of styrene (Gobba *et al*., 2000). Seven glass fibre-reinforced plastics workers not using respiratory protection devices were studied for a week. External exposure to styrene was evaluated by personal passive sampling and the internal dose by measurement of urinary styrene. Workers then wore half-mask respirators for a week and styrene exposure and internal dose were reassessed. Mean TWA concentrations of styrene for the morning half-shift for the first week ranged from 246.5 to 261.2 mg/m^3; mean levels for the second week ranged from 228.5 to 280.8 mg/m^3. Mean TWA concentrations of styrene for the afternoon half-shift for the first week ranged from 169.2 to 330.2 mg/m^3; mean levels for the second week ranged from 207.3 to 335.7 mg/m^3. Mean urinary concentrations of styrene for the first week ranged from 80.0 to 96.1 μg/L; for the second week, the means ranged from 31.5 to 47.2 μg/L. The authors concluded that the protection afforded by negative-pressure half-mask respirators varies widely, and stressed the need to assess the effective reduction of exposure whenever these devices are introduced for styrene-exposed workers. Measurement of urinary excretion of unmodified styrene was useful for the evaluation of respirator effectiveness in exposed workers.

Other substances may be found in workplace air in plants for the production of unsaturated polyester-reinforced plastics, although at levels usually considerably lower than that of styrene. These include: solvents, mainly used to clean tools and equipment, such as ketones (e.g., acetone), chlorinated hydrocarbons (e.g., dichloromethane), aliphatic alcohols and esters and aromatic hydrocarbons; organic peroxides used as initiators (e.g., methyl ethyl ketone peroxide, benzoyl peroxide); styrene 7,8-oxide and other oxidation products resulting from the reaction of peroxides with styrene; hydroquinone and analogues used as inhibitors (e.g., hydroquinone, quinone, catechol); dusts and fibres originating mainly from filler and reinforcement materials (e.g., glass fibres, silica, asbestos); foaming agents such as isocyanates; and cobalt salts and amines used as accelerators (Pfäffli *et al*., 1979; A.D. Little, Inc., 1981; Makhlouf, 1982; Högstedt *et al*., 1983; National Institute for Occupational Safety and Health, 1983; Coggon *et al*., 1987; Jensen *et al*., 1990; Bellander *et al*., 1994).

(*e*) *Miscellaneous operations*

In a study of exposures of firefighters, air samples taken during the ‘knockdown’ phase of a fire contained styrene at a concentration of 1.3 ppm [5.5 mg/m^3]; none was detected during the ‘overhaul’ phase (Jankovic *et al*., 1991). During working operations at a US hazardous waste site in 1983, a mean styrene concentration of 235 μg/m^3 (maximum, 678 μg/m^3) was measured in air for a group of workers nearest the areas where chemically contaminated materials were handled (Costello, 1983). During the manufacture of polyester paints, lacquers and putties in Finland, occasional high exposure to styrene was recorded, with 5% of measurements above 20 ppm [85 mg/m^3]; use of the

same products resulted in exposures below 1 ppm [4.3 mg/m^3] (Säämänen *et al*., 1991). Application of polyester putty during cable splicing operations for a telephone company in the USA resulted in short-term levels (3–16 min) ranging from 2 to 16 ppm [8.5–68 mg/m^3] in four samples (Kingsley, 1976). In a Japanese plant where plastic buttons were manufactured from polyester resins, the 8-h TWA concentration of styrene for 34 workers was 7.1 ppm [30 mg/m^3], with a maximum of 28 ppm [119 mg/m^3] (Kawai *et al*., 1992).

In four 100-min area air samples taken in 1982 at a college in the USA during a sculpture class in which polyester resins were used, styrene concentrations ranged from 0.8 to 1.2 ppm [3.4–5.1 mg/m^3]; two personal breathing zone air samples contained 2.8 and 3.0 ppm [11.9 and 12.8 mg/m^3]. The concentration of methyl ethyl ketone peroxide was below the detection limit (< 0.02 ppm) (Reed, 1983).

Taxidermists who used polyester resins during specimen preparation were shown to be exposed for short periods (2–34 min) to concentrations of styrene ranging from 21 to 300 mg/m^3 (12 samples) (Kronoveter & Boiano, 1984a,b).

In two cooking-ware manufacturing companies in the USA where styrene-based resins were used, the 8-h TWA concentrations of styrene ranged from 0.2 to 81 ppm [0.85–345 mg/m^3]; two short-duration samples (24 min) contained 142 and 186 ppm (605 and 792 mg/m^3] (Fleeger & Almaguer, 1988; Barsan *et al*., 1991).

1.4.3 *Environmental occurrence*

Human exposure to styrene has been assessed on the basis of a review of data in the published literature (Tang *et al*., 2000). The authors estimated that styrene exposure for the general population is in the range of 18.2–55.2 µg/person/day (0.3–0.8 µg/kg bw) or 6.7–20.2 mg/person/year (95.7–288 µg/kg bw), mainly resulting from inhalation and from food intake. The inhaled styrene accounts for more than 90% of the total intake. The styrene in food occurs mainly by migration from polymer packaging materials. The authors also concluded that cigarette smoking is another important source of styrene intake for smokers. The intake of styrene due to smoking 20 cigarettes was estimated to be higher than the total daily intake from food and air.

A Canadian study estimated a daily total styrene intake for the Canadian general population ranging from below 0.19 to over 0.85 µg/kg bw. Intakes from ambient air ranged from 0.004 up to 0.17 µg/kg bw and those from indoor air from 0.07 up to 0.10 µg/kg bw. Intake from food was calculated to range from below 0.11 to over 0.58 µg/kg bw. The estimated intakes from drinking water and soil were negligible. Potential exposure from cigarette smoke, on the basis of the styrene content reported for mainstream smoke (10 µg per cigarette) and a smoking rate of 20 cigarettes per day, was estimated to be 2.86 µg/kg bw per day for adults. The Canadian study estimated that styrene in food may represent a major exposure source for the general population (Health Canada, 1993; Newhook & Caldwell, 1993).

(*a*) *Air*

Styrene has been detected in the atmosphere in many locations. Its presence in air is due principally to emissions from industrial processes involving styrene and its polymers and copolymers. Other sources of styrene in the environment include vehicle exhaust, cigarette smoke and other forms of combustion and incineration of styrene polymers (WHO, 1983).

Styrene emissions reported to the European Union by member countries (Bouscaren *et al*., 1987) are shown in Table 6. Air emissions in the USA, reported to the US Environmental Protection Agency by approximately 1500 industrial facilities, increased from 15 600 tonnes in 1988 to 24 800 tonnes in 1999 (Environmental Protection Agency, 2001a). Based on Environmental Protection Agency (1999) national emission estimates, the total styrene emissions for 1990 can be allocated as follows: on-road vehicles, 17 900 tonnes (32.9% of total emissions); reinforced plastic composites production and boat manufacturing, 21 700 tonnes (39.8%); and all other sources, 14 900 tonnes (33.9%). Ambient air levels of styrene sampled in the vicinity (< 500 m) of seven reinforced plastic processors in three states in the USA ranged from 0.29 to 2934 μg/m^3, and those in communities near the processors (500–1000 m) from 1.67 to 23.8 μg/m^3 (McKay *et al*., 1982). Styrene levels of 1.1–6.6 μg/m^3 were measured in air samples from the Pennsylvania Turnpike Allegheny Mountain Tunnel in 1979. The mean concentration in the tunnel intake air was below 0.1 μg/m^3 (Hampton *et al*., 1983). Air concentrations of styrene in the vicinity of five rural hazardous waste sites in New Jersey, USA, ranged up to 66 μg/m^3 (LaRegina *et al*., 1986).

Table 6. Estimated emissions of styrene in member countries of the European Union (thousand tonnes per year)

Country	Source	
	Road traffic (gasoline)	Chemical industry
Belgium	0.5	0.75
Denmark	0.28	NR
France	2.9	3.4
Germany	2.9	3.4
Greece	0.5	NR
Ireland	0.19	NR
Italy	3.0	3.5
Luxembourg	0.02	0.03 (other sources)
Netherlands	0.7	1.45
Portugal	0.5	0.3
Spain	2.0	1.2
United Kingdom	3.0	3.7
Total	16.0	18.0

From Bouscaren *et al*. (1987); NR, not reported

Ambient air monitoring data from the USA include databases compiled and maintained by the California Air Resources Board. The 20 test stations are located in urban areas, representing the greatest portion of the California population. Styrene is measured on a 24-hour sample collected once each month. Based on data from the Board reflecting the measurements for each test station for each month from 1989 to 1995, the average reading for styrene was approximately 0.20 ppb [0.9 μg/m^3] over six years. The detection level for styrene was 0.1 ppb [0.4 μg/m^3] and the highest measurement was 2.9 ppb [12.4 μg/m^3] (Styrene Information & Research Center, 2001).

Styrene levels in ambient air were determined in a survey of 18 sites (mostly urban) in Canada in 1988–90. The mean concentrations in 586 24-h samples ranged from 0.09 to 2.35 μg/m^3. In a national survey of styrene levels in indoor air in 757 single-family dwellings and apartments, representative of the homes of the general population of Canada in 1991, the mean 24-h concentration was 0.28 μg/m^3, with values ranging from none detected (limit of detection, 0.48 μg/m^3) up to 129 μg/m^3 (Newhook & Caldwell, 1993).

For residential exposure, median concentrations obtained by personal air sampling are generally in the 1–3 μg/m^3 range (Wallace *et al*., 1985; Wallace, 1986). Exposure to styrene is approximately six times higher for smokers than for nonsmokers, and tobacco smoke is the major source of styrene exposure for smokers (Wallace *et al.*, 1987, 1989). Measurements in homes with and without smokers revealed that average styrene concentrations in the homes of smokers were approximately 0.5 μg/m^3 higher than those in the homes of nonsmokers. Hodgson *et al*. (1996) also found that environmental tobacco smoke (ETS) can contribute significantly to indoor airborne styrene concentrations. ETS was estimated to contribute 8% to the total styrene inhalation exposure of all nonsmoking Californians (Miller *et al.*, 1998).

Styrene is one of the hundreds of individual components that may be quantified in tobacco smoke (IARC, 1986; Darrall *et al.*, 1998; Health Canada, 1999; IARC, 2003). The styrene content of cigarette smoke has been reported to be 18–48 μg per cigarette (WHO, 1983). Off-gassing of styrene from some styrene-containing household products may also contribute to indoor air levels (Knöppel & Schauenburg, 1989).

In order to illustrate the relative significance of various sources of exposure to styrene, Fishbein (1992) estimated approximate exposure levels in several environments and compared nominal daily intakes from those sources (Table 7).

Thermal degradation of styrene-containing polymers also releases styrene into ambient air (Hoff *et al*., 1982; Lai & Locke, 1983; Rutkowski & Levin, 1986). Gurman *et al*. (1987) reported that styrene monomer is the main volatile product of the thermal decomposition of polystyrene, comprising up to 100% of the volatiles in special laboratory conditions.

Volatile organic compounds found at municipal structural fires have been characterized in order to identify sources of long-term health risks to firefighters. These compounds were identified and quantified using GC–MS in selected ion-monitoring mode. The compounds with the highest levels found were benzene, toluene and naphthalene

Table 7. Estimated intake of styrene from different sources of exposure

Source	Estimated concentration	Nominal daily intake[a]
Reinforced plastics industry	200 000 μg/m^3	2 g
Styrene polymerization	10 000 μg/m^3	100 mg
Within 1 km of a production unit	30 μg/m^3	600 μg
Polluted urban atmosphere	20 μg/m^3	400 μg
Urban atmosphere	0.3 μg/m^3	6 μg
Indoor air	0.3–50 μg/m^3	6–1000 μg
Polluted drinking-water (2 L/day)	1 μg/L	2 μg
Cigarette smoke (20 cigarettes/day)	20–48 μg/cigarette	400–960 μg

From Fishbein (1992)

[a] Calculated on the assumption of a daily respiratory intake of 10 m^3 at work or 20 m^3 at home or in an urban atmosphere

(see monograph in this volume); styrene and other alkyl-substituted benzene compounds were frequently identified (Austin *et al*., 2001).

Little is known about factors that influence blood levels of volatile organic compounds in non-occupationally exposed populations. Possible relationships were examined between recent self-reported chemical exposures and elevated blood volatile organic compound levels among 982 adult participants in the Third National Health and Nutrition Examination Survey in the USA. A strong relationship was found ($p < 0.001$) between increasing lifetime pack–years of cigarettes smoked and elevated levels of toluene, styrene, and benzene (Churchill *et al*., 2001).

(*b*) *Water*

Although styrene has been detected occasionally in estuaries and inland waters and in drinking-water, its presence is usually traceable to an industrial source or to improper disposal (WHO, 1983; Law *et al*., 1991). In surveys of Canadian drinking-water supplies, the frequency of detection of styrene was low; when detected, it was generally at a concentration below 1 μg/L (Newhook & Caldwell, 1993). After accidental drinking-water contamination with styrene in Spain, transient levels up to 900 μg/L were reported (Arnedo-Pena *et al.*, 2002).

(*c*) *Food*

Polystyrene and its copolymers have been used widely as food packaging materials, and residual styrene monomer can migrate into food from such packaging (WHO, 1983). Analysis of styrene in 133 plastic food containers from retail food outlets in the United Kingdom showed concentrations ranging from 16 to 1300 mg/kg; 73% of containers had styrene concentrations of 100–500 mg/kg, and only five containers had levels exceeding 1000 mg/kg. The food in the containers had levels of monomer ranging from below 1 to

200 µg/kg, although 77% of the foods had levels below 10 µg/kg and 26% had levels below 1 µg/kg (Gilbert & Startin, 1983).

Similar surveys were carried out by the Food Safety Directorate in 1992 and 1994 in the United Kingdom, with styrene concentrations similar to those found in the 1983 survey. Within each food type, higher levels of styrene were generally found for products with high fat content or packed in small containers (Ministry of Agriculture, Fisheries and Food, 1994).

Between 1991 and 1999, the Food and Drug Administration's Total Diet Study in the USA analysed 320 different foods and found styrene residues in 49 of them. In 258 samples containing styrene, the mean concentrations for individual food items varied between 10 µg/kg (eggs) and 274 µg/kg (strawberries). The median concentration for the 49 foods was 21 µg/kg (Food and Drug Administration, 2000).

Several assessments have been made of estimated daily intake (EDI) of styrene in food packaged in polymers or copolymers of styrene. In 1981, the Food and Drug Administration in the USA measured the migration of styrene over a 24-h period from foam, impact and crystal polystyrene cups into 8% ethanol and water at 49 °C. The observed migration was 0.036, 0.064 and 0.210 µg/cm^2, respectively. This corresponds to 6 µg styrene from a 2 dL (8 oz.) foam cup. Migration from foam cups into hot water, tea and coffee was one fifth of these levels: approximately 1 µg from a 2 dL (8 oz.) cup (Varner & Breder, 1981). In a 1983 study, the EDI of styrene monomer from polystyrene food packaging, including polystyrene foam cups, was in the range of 1 to 4 µg per day (Ministry of Agriculture, Fisheries and Food, 1989). More recently, Lickly *et al.* (1995) estimated total dietary intake of styrene monomer from polystyrene food-contact polymers to be 9 µg per day.

Styrene has been detected at low levels (ppb) in several foods and beverages which had not previously been in contact with styrene-containing packaging materials (Maarse, 1992a,b; Steele, 1992, Steele *et al.*, 1994). Much higher levels (around 40 mg/kg) have been measured in cinnamon, and enzymatic degradation of cinnamic acid derivatives was proposed as a possible source (Oliviero, 1906; Ducruet, 1984). The low-level occurrence of styrene in other foods has been suggested to result from enzymatic and/or microbial activity, but it is unclear to what extent these processes are, in fact, responsible for the levels detected (Steele *et al.*, 1994; Tang *et al.*, 2000).

1.5 Regulations and guidelines

1.5.1 *Exposure limits and guidelines*

Occupational exposure limits and guidelines for styrene are presented in Table 8. A tolerable daily intake (TDI) of 7.7 µg/kg bw for styrene has been established by WHO (1993), with a guideline value of 20 µg/L in drinking-water. The Environmental Protection Agency (2001b) has set a maximum contaminant level (MCL) for styrene in public water systems in the USA at 0.1 mg/L.

The Food and Drug Administration (2001) has established regulations for the use of polymers and copolymers of styrene in products in contact with food in the USA. For styrene and methyl methacrylate copolymers as components of paper and paperboard in contact with fatty foods, the monomer content in the copolymer is limited to 0.5%. For styrene–acrylic copolymers, the level of residual styrene monomer in the polymer should not exceed 0.1% by weight.

Table 8. Occupational exposure limits and guidelines for styrene

Country or region	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	213	TWA
		426	STEL
Belgium	1993	213 (sk)	TWA
		426	STEL
Czech Republic	1993	200	TWA
		1000	STEL
Denmark	1993	106	TWA
Finland	2002	86	TWA
		430	STEL
France	1993	213	TWA
Germany	2001	86 (category 5)[a]	MAK; substance with systemic effects (onset < 2 h)
Hungary	1993	50 (Ca)	TWA
Ireland	1993	426	TWA
		1065	STEL
Japan	2000	85 (sk)	TWA (provisional value)
Mexico	1984	215	TWA
		425	STEL
Netherlands	1999	106	TWA
Philippines	1993	426	TWA
Poland	1998	50 (sk)	TWA
		200	STEL
Sweden	1993	106 (sk)	TWA
		320	STEL
Switzerland	1993	213	TWA
		426	STEL
Thailand	1993	426	TWA
		852	STEL
Turkey	1993	426	TWA
United Kingdom	2000	430	TWA; maximum exposure limit
		1065	STEL

Table 8 (contd)

Country or region	Year	Concentration (mg/m^3)	Interpretation
USA			
ACGIH (TLV)	2001	85 (A4, ir)	TWA
		170	STEL
OSHA (PEL)	2001	426	TWA
		852	Ceiling
NIOSH (REL)	2000	213	TWA
		426	Ceiling

From American Conference of Governmental Industrial Hygienists (ACGIH) (2000, 2001); Deutsche Forschungsgemeinschaft (2001); Occupational Safety and Health Administration (OSHA) (2001); Sosiaali-ja terveysministeriö (2002); United Nations Environment Program (UNEP) (2002)

TWA, 8-h time-weighted average; STEL, short-term exposure limit; MAK, maximum workplace concentration; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level; A4, not classifiable as a human carcinogen; Ca, suspected of having carcinogenic potential; ir, irritant; sk, absorption through the skin may be a significant source of exposure

[a] Category 5, substances with carcinogenic and genotoxic potential, the potency of which is considered to be so low that, provided that the MAK value is observed, no significant contribution to human cancer risk is to be expected

1.5.2 *Reference values for biological monitoring of exposure*

The relationship between external (air concentrations) and biological measures of exposure has been studied more extensively for styrene than for most other organic compounds in the occupational environment. Various reported correlations between the concentration of styrene in air and those in venous blood and with mandelic acid and phenylglyoxylic acid levels in urine have been reviewed by Guillemin and Berode (1988), Lauwerys and Hoet (1993), Pekari *et al*. (1993) and the American Conference of Governmental Industrial Hygienists (2001).

For example, the concentration of mandelic acid in urine that corresponds to inhalation of 50 ppm styrene (213 mg/m^3) for 8 h would be approximately 800–900 mg/g creatinine at the end of a shift and 300–400 mg/g creatinine the following morning (Droz & Guillemin, 1983; Guillemin & Berode, 1988; Pekari *et al*., 1993). The phenylglyoxylic acid concentration in urine that corresponds to an 8-h exposure to 50 ppm styrene would be expected to be 200–300 mg/g creatinine at the end of a shift and about 100 mg/g creatinine the following morning (Pekari *et al*., 1993; American Conference of Governmental Industrial Hygienists, 2001). The styrene concentration in blood that corresponds to an 8-h exposure to 50 ppm styrene would be expected to be 0.5–1 mg/L

at the end of a shift and about 0.02 mg/L in blood the following morning (Guillemin & Berode, 1988; American Conference of Governmental Industrial Hygienists, 2001).

Each year, the American Conference of Governmental Industrial Hygienists (ACGIH) (2001) and the Deutsche Forschungsgemeinschaft (2001) publish biological reference values for use in interpreting the results of biological monitoring for styrene in the workplace. The results must be interpreted in relation to the different definitions of those reference values. ACGIH biological exposure indices are reference values intended for use as guidelines for evaluating potential health hazards in the practice of industrial hygiene. The indices represent the levels of the determinants that are most likely to be observed in specimens collected from healthy workers exposed by inhalation to air concentrations at the level of the threshold limit value (American Conference of Governmental Industrial Hygienists, 2001). In Germany, the biological tolerance value (BAT) for occupational exposures is defined as the maximal permissible quantity of a chemical compound or its metabolites, or the maximum permissible deviation from the norm of biological parameters induced by those substances in exposed humans. According to current knowledge, these conditions generally do not impair the health of an employee, even if exposure is repeated and of long duration. The BAT values are conceived as ceiling values for healthy individuals (Deutsche Forschungsgemeinschaft, 2001).

Biological monitoring reference values for exposure to styrene, based on styrene metabolite levels in urine or styrene in blood, are given in Table 9.

Table 9. Reference values for biological monitoring of exposure to styrene

Determinant	Sampling time	Biological exposure index[a]	BAT[b]
Mandelic acid in urine	End of shift	800 mg/g creatinine[c]	Does not apply
	Prior to next shift	300 mg/g creatinine[c]	Does not apply
Phenylglyoxylic acid in urine	End of shift	240 mg/g creatinine[c]	Does not apply
	Prior to next shift	100 mg/g creatinine[c]	Does not apply
Mandelic acid plus phenylglyoxylic acid in urine	End of shift	Does not apply	600 mg/g creatinine
Styrene in venous blood	End of shift	0.55 mg/L[d]	Does not apply
	Prior to next shift	0.02 mg/L[d]	

[a] American Conference of Governmental Industrial Hygienists (2001)
[b] BAT, Biologischer Arbeitsstoff-Toleranz-Wert (biological tolerance value for occupational exposures) (Deutsche Forschungsgemeinschaft, 2001)
[c] Non-specific, as it is also observed after exposure to other chemicals such as ethylbenzene
[d] Semiquantitative, because of short half-life of styrene in blood

2. Studies of Cancer in Humans

2.1 Case reports

Cases of leukaemia and lymphoma were identified among workers engaged in the production of styrene–butadiene rubber (Lemen & Young, 1976), in the manufacture of styrene–butadiene (Block, 1976) and in the manufacture of styrene and polystyrene (Nicholson *et al*., 1978). A total of 16 cases of leukaemia and 9 of lymphoma were reported in these studies. In addition to styrene, exposure to benzene, 1,3-butadiene, ethylbenzene and other chemicals could have occurred in these operations.

2.2 Cohort studies (see Table 10)

2.2.1 *Styrene manufacture and polymerization*

A study by Frentzel-Beyme *et al*. (1978) of 1960 workers engaged in the manufacture of styrene and polystyrene in Germany between 1931 and 1976 showed no significant excess mortality from cancer. The cohort had accumulated 20 138 person–years. Follow-up was 93% for the German workers but only 29% for the non-German workers and there were only 74 deaths (96.5 expected) available for analysis. Only one death from lymphatic cancer was observed. There were two deaths from pancreatic cancer (0.7 expected). In 1975 and 1976, concentrations of styrene in the plant were generally below 1 ppm [4.3 mg/m^3], but higher concentrations were occasionally recorded (Thiess & Friedheim, 1978). [The Working Group noted that insufficient information was provided to assess the risk for cause-specific deaths by exposure period or duration of exposure.]

Ott *et al*. (1980) studied a cohort of workers at four plants in the USA where styrene-based products were developed and produced. Exposure to styrene varied by process and time period. During container filling for batch polymerization in 1942, styrene concentrations ranged from 5 to 88 ppm [21–375 mg/m^3]; in continuous polymerization and extrusion units, the concentrations were below 10 ppm [43 mg/m^3] and generally below 1 ppm in 1975 and 1976. Cohorts from each plant had been exposed between 1937 and 1970 and were followed from 1940 to 1975. Other potential exposures included benzene, acrylonitrile, 1,3-butadiene, ethylbenzene, dyes and pigments. Age- and race-specific US mortality rates were used to calculate the expected numbers of deaths. A total of 2904 workers with a minimum of one year of employment were included. Bond *et al*. (1992) updated the study, adding a further 11 years of follow-up. Based on this update, the standardized mortality ratio (SMR) for all causes was 0.76 (95% confidence interval [CI], 0.70–0.82; 687 deaths), for all cancers was 0.81 (95% CI, 0.69–0.95; 162 deaths) and for lymphatic and haematopoietic malignancies was 1.4 (95% CI, 0.95–2.1; 28 deaths). The excess of lymphatic and haematopoietic neoplasms was restricted to

Table 10. Characteristics of cohort and nested case–cohort studies of incidence or mortality from neoplasms among workers exposed to styrene

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No of deaths/ cancer deaths	Results				Comments (previous evaluation)
			No.	SMR/ SIR	95% CI	Site	
Frentzel-Beyme *et al.* (1978) (Germany)	Styrene and polystyrene manufacture facility; 1931–76; 1960 subjects; 1 month; 93% among German, 29% among non-German	74/11	1	–	–	Lymphoma	Lymphoma case was a malignant neoplasm of the spleen (IARC, 1994a). Expected number based on the statistics for the town of Ludwigshafen during 1970–75 was 0.06. Two pancreatic cancer deaths were observed, 0.7 expected, respectively; and three lung cancer deaths, 5.7 expected.
Ott *et al.* (1980) (USA)	Dow Chemical workers in development or production of styrene-based products; 1940–75; 2904 men; 1 year; 97.0%	303/58	12 21	1.6 1.6	0.84–2.8 1.0–2.5	L&H, mortality L&H, incidence	Excess incidence of lymphatic leukaemia (SIR, 4.3; 95% CI, 1.7–8.8; 7 cases); highest risks in workers exposed to styrene, ethylbenzene, other fumes, solvents and colourants (IARC, 1994a)
Hodgson & Jones (1985) (United Kingdom)	Production, polymerization and processing of styrene; 1945–78; 622 men; 1 year; 99.7% among exposed, 94.4% among referents	34/10 (exposed)	5 3 0 4	[1.2] [5.4] – [2.5]	[0.39–2.8] [1.1–16] – [0.67–6.4]	Lung, mortality Lymphomas, mortality Leukaemias, mortality L&H, incidence	Exposure to styrene among other chemicals such as 1,3-butadiene, acrylonitrile, benzene, dyestuff, and ethylene oxide (IARC, 1994a)
Okun *et al.* (1985) (USA)	Reinforced plastics boat building (two facilities); 1959–78; 5021 subjects; 1 day; 98.1%	176/36	16 0	1.4 0	[0.82–2.3] [0–0.88]	Lung L&H	From L&H, 4.2 deaths expected in the whole cohort, and about one death expected within the group with high exposure to styrene (IARC, 1994a)

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No of deaths/ cancer deaths	Results				Comments (previous evaluation)
			No.	SMR/ SIR	95% CI	Site	
Coggon *et al.* (1987) (United Kingdom)	Production of glass-reinforced plastics (8 facilities); 1947–84; 7949 subjects; no minimal employment; variable, 61.9–99.7%	693/181	6	[0.40]	[0.15–0.88]	L&H, mortality	One of six deaths from L&H with high exposure to styrene. Extended follow-up of this cohort included by Kogevinas *et al.* (1994a,b) (IARC, 1994a)
Bond *et al.* (1992) (USA)	Dow Chemical workers in development or production of styrene-based products; 1940–86; 2904 men; 1 year; 96.7%	687/162	15	0.86	0.48–1.4	Large intestine	A mortality study, updating of Ott *et al.* (1980). From among L&H cancers, elevated incidences of multiple myeloma and Hodgkin disease (IARC, 1994a)
			2	[0.39]	0.04–1.4	Rectum	
			5	0.49	0.16–1.1	Pancreas	
			56	0.81	0.61–1.0	Lung	
			6	0.95	0.35–2.1	Brain/other nervous system	
			28	1.4	0.95–2.1	L&H	
Kolstad *et al.* (1993, 1994, 1995) (Denmark)	Production of glass fibre-reinforced plastics and other plastics (552 facilities); 1970–89; 53 720 men and 10 798 women; no minimal employment; 98.2%	4281/2285 incident cases	174	[1.1]	[0.93–1.3]	L&H, men/women	Significantly higher incidence of leukaemia (SIR, 1.6) among men at ≥ 10 years since first exposure; incidence of leukaemia higher among short-term male workers with estimated higher exposure to styrene; part of this cohort included by Kogevinas *et al.* (1994a,b) (IARC, 1994a)
			66	[1.1]	[0.86–1.4]	Leukaemia, men/women	
			112	1.2	0.98–1.4	L&H, men[a]	
			42	1.2	0.88–1.7	Leukaemia, men[a]	
			90	0.87	0.70–1.1	Breast, women	
			16	1.2	0.68–1.9	Brain and nervous system, women	

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No of deaths/ cancer deaths	Results				Comments (previous evaluation)
			No.	SMR/ SIR	95% CI	Site	
Kogevinas *et al.* 1994a,b) (six European countries)	Production of glass fibre-reinforced plastics (660 facilities); 1945–91; varies between cohorts; 40 688 workers; 97%	2714/686	39	0.77	0.55–1.1	Colon	Risk of L&H increased with latency (p for trend = 0.012) and with average exposure (p for trend = 0.019); risk did not increase with duration of exposure or cumulative exposure. Non-significant excess risk observed for pancreas for high cumulative exposure to styrene (p for trend = 0.068) (IARC, 1994a). Results presented in this table are for the full cohort which included a non-exposed group.
			21	0.62	0.38–0.95	Rectum	
			37	1.0	0.71–1.4	Pancreas	
			235	0.99	0.87–1.1	Lung	
			18	0.62	0.37–0.98	Brain	
			60	0.93	0.71–1.2	L&H	
			28	1.0	0.69–1.5	Leukaemia	
Wong *et al.* (1994) (USA)	Reinforced plastics manufacturing plants (n = 30); 1948–89; 15 826 subjects; 6 months; 96.5%	1628/425	36	1.2	0.83–1.6	Large intestine	Higher risk of L&H among workers with cumulative exposure > 100 ppm–years at > 20 years since first exposure (SMR, 1.3; 5 deaths); SMR of lung cancer among workers with cumulative exposure > 100 ppm–years, 1.0 (34 deaths) (IARC, 1994a)
			19	1.1	0.68–1.8	Pancreas	
			162	1.4	1.20–1.6	Bronchus, trachea, lung	
			14	0.62	0.34–1.1	Breast	
			8	0.59	0.25–1.2	Central nervous system	
			31	0.82	0.56–1.2	L&H	
			11	0.74	0.37–1.3	Leukaemia and aleukaemia	
Anttila *et al.* (1998) (Finland)	Database of workers biologically monitored for urinary mandelic acid; 1973–92; 2580 subjects; no minimal employment; 98.5%	NR/48	1	0.36	0.01–2.0	Colon	Both of the L&H cases were Hodgkin lymphomas.
			6	3.1	1.14–6.8	Rectum	
			3	1.7	0.34–4.9	Pancreas	
			5	0.59	0.19–1.4	Lung, trachea	
			5	0.62	0.20–1.5	Breast	
			6	1.6	0.59–3.5	Nervous system	
			2	0.39	0.05–1.4	L&H	

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No of deaths/ cancer deaths	Results				Comments (previous evaluation)
			No.	SMR/ SIR	95% CI	Site	
Sathiakumar *et al.* (1998) (USA and Canada)	Styrene–butadiene rubber plants (n = 8); 1943–91; 15 649 men; one year; 95%	3967/950	87 20 43 349 25 101 48 9	0.97 0.78 0.82 1.01 0.92 1.1 1.3 0.82	0.78-1.2 0.48-1.2 0.60-1.1 0.91-1.1 0.59-1.4 0.88-1.3 0.97-1.7 0.37-1.6	Large intestine Rectum Pancreas Lung Central nervous system L&H Leukaemia Benign neoplasms	Among ever hourly subjects with at least ten years worked and 20 years since hire: significantly increased mortality from L&H and leukaemia (SMRs, 1.5 and 2.2; 49 and 28 deaths)
Loughlin *et al.* (1999) (USA)	Students attending a school adjacent to a styrene–butadiene facility; 1963–95; 15 403 students, 7882 men, 7521 women; 3 months; NR	338/44	6 14 7 6	[1.1] [1.2] [1.3] [4.2]	[0.37–2.2] [0.66–2.0] [0.51–2.6] [1.5–9.1]	Lung L&H Leukaemia Benign neoplasms	No data on specific environmental exposures to chemicals among school students
Delzell *et al.* (2001) (USA and Canada)	Styrene–butadiene rubber plants (n = 6); 1943–91; 13 130 men; one year; vital status known for > 99%, death certificates for 98% of the decedents	3892/NR	59 59	3.2 0.8	1.2–8.8 0.2–3.8	Leukaemia (unadjusted) Leukaemia (adjusted)	Within-cohort comparison; the cohort follow-up reported in Sathiakumar *et al.* (1998). Relative risks for the high cumulative exposure to styrene (18 exposed cases); whether unadjusted, or adjusted for exposure to 1,3-butadiene and DMDTC; both models included also terms for age and years since hire.

L&H, malignancies of the lymphatic and haematopoietic tissues; NR, not reported; SIR, standardized incidence ratio; SMR, standardized mortality ratio; CNS, central nervous system; DMDTC, dimethyldithiocarbamate

[a] Only men in the companies producing reinforced plastics

workers with less than five years of employment and was significantly increased among workers after 15 years of follow-up (SMR, 1.6; 95% CI, 1.0–2.4).

Hodgson and Jones (1985) reported on 622 men who had worked for at least one year in the production, polymerization and processing of styrene at a plant in the United Kingdom between 1945 and 1974 who were followed until 1978. Of these, 131 men were potentially exposed to styrene in laboratories and 491 in production of styrene monomer, polymerization of styrene or manufacture of finished products. No measurements of exposure were provided, but many other chemicals were present in the working environment. Expected numbers of deaths were calculated on the basis of national rates. There were 34 deaths (43.1 expected) among the 622 exposed workers. A significant excess of deaths from lymphoma (SMR, 5.4; [95% CI, 1.1–16]; 3 deaths) was observed. An analysis of cancer registrations for this population revealed an additional case of lymphatic leukaemia, giving a total of four incident cases of lymphatic and haematopoietic cancer, whereas 1.6 would have been expected from local cancer registration rates [standardized incidence ratio (SIR), 2.5; 95% CI, 0.67–6.4]. In addition, three incident cases of laryngeal cancer were found (0.5 expected; [SIR, 6.0; 95% CI, 1.2–18]).

2.2.2 *Use of styrene in reinforced plastics*

Okun *et al*. (1985) studied 5021 workers who had been employed in two reinforced-plastic boat-building facilities in the USA for at least one day between 1959 and 1978. On the basis of industrial hygiene surveys, 2060 individuals were classified as having had high exposure to styrene, with means in the two facilities of 42.5 and 71.7 ppm [181 and 305 mg/m^3]. Of these, 25% had worked for less than one month, 49% had worked for one month to one year and only 7% had worked for more than five years. There were 47 deaths in the high-exposure group (41.5 expected); no cases of lymphatic or haematopoietic cancer were observed in the high-exposure group (approximately one expected) or in the full cohort (4.2 expected).

Wong (1990) and Wong *et al*. (1994) reported on a cohort of 15 826 male and female employees who had worked at one of 30 reinforced-plastics plants in the USA for at least six months between 1948 and 1977. Workers were followed until 1989; vital status was determined using Social Security Administration files, the National Death Index and the records of credit agencies. A total of 307 932 person–years at risk were accumulated. Expected numbers of deaths were based on national age-, gender-, cause- and year-specific death rates for whites, as no information was available on race. Exposure to styrene was calculated using a job–exposure matrix that included work history and current and past time-weighted average exposures. A total of 1628 (10.3%) members of the cohort were found to have died, and death certificates were obtained for 97.4% of them. The overall SMR was 1.08 (95% CI, 1.03–1.13) and the SMR from all cancers was 1.16 (95% CI, 1.05–1.27). Mortality from cancers at a number of sites was increased significantly; the SMRs for these sites were: oesophagus, 1.9 (95% CI, 1.1–3.2; 14 deaths); bronchus, trachea and lung, 1.4 (95% CI, 1.2–1.6; 162 deaths); cervix uteri, 2.8

(95% CI, 1.4–5.2; 10 deaths); and other female genital organs, 2.0 (95% CI, 1.1–3.5; 13 deaths). In the cohort as a whole, no excess was observed for lymphohaematopoietic cancers (SMR, 0.82; 95% CI, 0.56–1.2; 31 deaths), nor was there a strong suggestion of excess risk for any of these cancers in any of the subgroups analysed. The data were analysed by Cox regression with age, gender, length of exposure and cumulative exposure included in the model. Neither cumulative exposure nor length of exposure was significantly related to risk in the model for lymphatic and haematopoietic cancer. No positive dose–response relationship was found for any other cancer that was analysed. [The Working Group noted that the possibility that the inclusion of two correlated indices of styrene exposure in the regression models may have artificially reduced the coefficients of both.]

Kogevinas *et al.* (1993, 1994a,b) included an extended follow-up of the study by Coggon *et al.* (1987) and parts of the material of Kolstad *et al.* (1993, 1994, 1995) and Härkönen *et al.* (1984). The study included 40 688 workers employed in 660 plants of the reinforced plastics industry and enrolled in eight subcohorts in Denmark, Finland, Italy, Norway, Sweden and the United Kingdom. Exposure to styrene was reconstructed from job and production records, environmental measurements and, in Italy, biological monitoring. An exposure database was constructed on the basis of about 16 400 personal air samples and 18 695 measurements of styrene metabolites in urine. Styrene exposure levels decreased considerably during the study period. The data from Denmark, considered to be representative of all six countries, showed exposures of about 200 ppm [852 mg/m^3] in the early 1960s, about 100 ppm [430 mg/m^3] in the late 1960s and about 20 ppm [85 mg/m^3] in the late 1980s. The 40 688 workers accumulated 539 479 person–years at risk and were followed for an average of 13 years. Workers lost to follow-up and those who emigrated constituted 3.0% of the total cohort, and in no individual cohort did this proportion exceed 8.0%; 60% of the cohort had less than two years' exposure and 9% had more than 10 years' exposure. The WHO mortality data bank was used to compute national mortality reference rates by sex, age (in five-year groups) and calendar year. No excess was observed for mortality from all causes [SMR, 0.96; 95% CI, 0.92–1.00; 2196 deaths] or from all neoplasms (SMR, 0.91; 95% CI, 0.83–0.99; 536 deaths) among styrene-exposed workers. The SMR for malignant neoplasms was 0.91 (95% CI, 0.78–1.06; 167 deaths) among laminators and 0.73 (95% CI, 0.59–0.88; 106 deaths) among unexposed workers. The mortality rate in exposed workers for neoplasms of the lymphatic and haematopoietic tissues was not elevated (SMR, 0.98; 95% CI, 0.72–1.3; 49 deaths) and was not associated with length of exposure. When the duration of exposure was two years or more and at least 20 years had elapsed since first exposure, the SMR for all lymphatic and haematopoietic cancers was 1.7 (95% CI, 0.70–3.6; seven deaths) and that for leukaemia was 1.9 (95% CI, 0.40–5.7; three deaths). Evaluation of risk by job type showed no meaningful pattern. In an analysis by country, one of the cohorts in the United Kingdom and that in Denmark had moderately increased mortality from lymphatic and haematopoietic cancer. There was no significant increase in the SMRs for other cancers; there was a nearly significant increase in risk for pancreatic

cancer among workers in the highest exposure category (≥ 500 ppm-years) (SMR, 2.6; 95% CI, 0.90–7.3; 10 deaths). [The Working Group noted that the overall SMR for the unexposed group was very low, possibly reflecting problems in ascertaining mortality in some subcohorts. This may have negatively biased the SMRs in this study.] In contrast with the results of SMR analyses using the external referents, the results of internal analyses did show some statistically significant elevations in risk related to exposure to styrene. An increased risk for lymphatic and haematopoietic cancers was observed in Poisson regression models for average exposure (p = 0.019), culminating in a relative risk of 3.6 (95% CI, 1.0–13; n = 8) for the highest category, > 200 ppm. A statistically significant trend (p = 0.012) was also observed for time since first exposure, the relative risk reaching a peak after 20 years of 4.0 (95% CI, 1.3–12; n = 9). No trend was observed in the Poisson regression analysis with cumulative exposure or duration of exposure.

In the study by Kolstad *et al.* (1993, 1994), 64 529 workers (53 731 men and 10 798 women) in 552 companies engaged in production of reinforced plastics were followed up from 1970 to the end of 1989 through the Danish national cancer registry and the national mortality database. [The Working Group noted that the females were excluded from the analysis in the second paper by Kolstad *et al.* (1994) because 'the majority were not involved in the production of reinforced plastics'.] Information on the companies' activities was obtained from two dealers. Altogether, 386 companies were classified as ever producing reinforced plastics (with 36 525 male workers) and 84 were classified as never producing (with 14 254 male workers); for 82 companies, the information was unknown. All 12 837 men and 2185 women from 287 plants where the main product was reinforced plastics (involving more than 50% of the workforce in the plant) were included in the study by Kogevinas *et al.* (1993, 1994a,b). Fifty-three persons had disappeared from the follow-up and, in addition, 1104 had emigrated during the study period; the total loss to follow-up was 1.9%. A total of 584 556 person–years were accumulated until death, emigration, disappearance or the end of the study (whichever came first). The mean annual levels of styrene exposure calculated for 128 of these companies reflect the exposures measured in the industry, which ranged from 180 ppm [767 mg/m^3] in 1964–70 to 43 ppm [183 mg/m^3] in 1976–88. Duration of employment was calculated from pension fund payments made beginning in 1964–89. However, in a small validation sub-study of 671 workers, the authors found errors in the records which led to underestimation of duration of employment in the industry for about 40% of the workers and to overestimation for about 13%. There were too few women to provide statistically stable results. Among men, there were a total of 4281 deaths in the cohort and 1915 incident cases of cancer (SIR, 1.02; 95% CI, 0.97–1.07). Within companies producing reinforced plastics, there were slight increases in risk for lymphatic and haematopoietic cancers (SIR, 1.2; 95% CI, 0.98–1.4; 112 cases) and for leukaemia (SIR, 1.2; 95% CI, 0.88–1.7; 42 cases). A statistically significant increased risk for leukaemia was found after 10 years since first employment (SIR, 1.6; 95% CI, 1.1–2.2; 32 cases); however, the risk was confined to workers employed for less than one year (SIR, 2.3; 95% CI, 1.4–3.6; 20 cases). A significant increase in the incidence of leukaemia was observed for those

who had been employed in 1964–70 (the period with the highest exposure to styrene) (SIR, 1.5; 95% CI, 1.0–2.2; 30 cases). [The Working Group noted that the misclassification of duration of employment that was noted by the authors would also have applied to the Danish component of the European study.]

Kolstad *et al.* (1995) also published results on solid cancers (not including lymphatic and haematopoietic cancers) among men within the reinforced plastics industry in Denmark. The study cohort was essentially the same as that in the previous studies (Kolstad *et al.*, 1993; Kogevinas *et al.*, 1994a,b; Kolstad *et al.*, 1994). There were 36 310 male workers from the reinforced plastics companies, and 14 293 workers in similar industries not producing reinforced plastics (127 more workers than in the previous studies). Altogether, 1134 solid cancers were observed during 1970–89 within the reinforced plastics industry (SIR, 0.99; 95% CI, 0.93–1.1); there were 47 cases of rectal cancer (SIR, 0.78; 95% CI, 0.58–1.0), 41 cases of pancreatic cancer (SIR, 1.2; 95% CI, 0.86–1.6) and 46 cases of tumours of the brain and nervous system (SIR, 0.97; 95% CI, 0.71–1.3). The relative risk for pancreatic cancer incidence was 2.2 (95% CI, 1.1–4.5; 17 cases) for employment with a high probability of exposure to styrene, and duration of employment at least one year, compared with no exposure.

2.2.3 *Styrene–butadiene rubber manufacture*

A number of reports have presented results on the mortality of workers in the styrene–butadiene synthetic rubber (SBR) industry (McMichael *et al.*, 1976a,b; Meinhardt *et al.*, 1982; Matanoski *et al.*, 1990; Santos-Burgoa *et al.*, 1992; Matanoski *et al.*, 1993). With some exceptions, these workers were included in the University of Alabama study. These earlier studies suggested an increased risk for lymphatic and haematopoietic malignancies in the SBR industry, but generally did not provide data on exposure to styrene *per se* and are far less informative than the more recent investigations from the University of Alabama, which are described below.

A study performed by researchers at the University of Alabama (Delzell *et al.*, 1996; Sathiakumar *et al.*, 1998) assessed the mortality experience of 15 649 male synthetic rubber workers employed for at least one year at eight SBR plants in the USA and Canada (information concerning exposure to 1,3-butadiene was summarized in IARC (1999)). A two-plant complex had been previously studied by Meinhardt *et al.* (1982) and the other six plants by Matanoski *et al.* (1990, 1993) and Santos-Burgoa *et al.* (1992). Complete work histories were available for 97% of the subjects. During 1943–91, the cohort had a total of 386 172 person–years of follow-up. Altogether 10 939 (70%) of the subjects were classified as being alive, 3976 (25%) were deceased and 734 (5%) were lost to follow-up. Information on cause of death was available for 97% of decedents. The observed total of 3976 deaths compared with 4553 deaths expected on the basis of general population mortality rates for the USA and Ontario (SMR, 0.87; 95% CI, 0.85–0.90). Cancer mortality was slightly lower than expected, with 950 deaths (SMR, 0.93; 95% CI, 0.87–0.99). Lymphopoietic cancers accounted for 101 deaths, slightly

more than the number expected (SMR, 1.1; 95% CI, 0.88–1.3). There were 48 observed deaths from leukaemia in the overall cohort (SMR, 1.3; 95% CI, 0.97–1.7). There was a statistically significant excess of leukaemia among workers in polymerization (15 deaths; SMR, 2.5; 95% CI, 1.4–4.1), maintenance labour (13 deaths; SMR, 2.7; 95% CI, 1.4–4.5) and laboratories (10 deaths; SMR, 4.3; 95% CI, 2.1–7.9), which were three areas with potential for relatively high exposure to 1,3-butadiene or styrene monomers. Among the 'ever hourly-paid' workers with 10 or more years of employment and 20 or more years since hire, there was a significant excess of leukaemia deaths (28 deaths; SMR, 2.2; 95% CI, 1.5–3.2).

Delzell *et al.* (2001) and Sielken & Valdez-Flores (2001) re-analysed the University of Alabama results on leukaemia deaths within the US and Canadian cohorts. In these studies, the exposure assessments for 1,3-butadiene and styrene were revised, as compared with the earlier report (Macaluso *et al*., 1996) and exposure estimates were developed for dimethyldithiocarbamate (DMDTC). [The Working Group noted that, unlike in the earlier report, assessment of exposure was performed after knowing the jobs and departments of the leukaemia cases.] Workers from two facilities had exposure records of insufficient quality and were dropped from the original study, leaving 13 130 men from six of the plants, and 59 deaths from leukaemia (medical records were obtained for 48 cases and one case was an acute unspecified leukaemia) during 1943–91 (in 234 416 person–years). Vital status was known for over 99% of the subjects, and death certificates were available for 98% of the decedents. In the within-cohort comparisons (Poisson regression), unlike in the cohort (SMR) study, all leukaemia deaths (leukaemia being either an underlying or a contributing cause of death) were used; 49 of the leukaemia deaths were confirmed from medical records. About 79% of the cohort subjects were exposed to 1,3-butadiene, the median cumulative exposure being 71 ppm–years [301 mg/m^3–years], and 85% to styrene, with a median cumulative exposure of 17 ppm–years [72 mg/m^3–years], and 62% to DMDTC with a median cumulative exposure[1] of 373.9 mg–years/cm. Poisson regression analyses with the individual exposures indicated a positive and monotonically increasing association between grouped cumulative exposure to styrene (relative risks of 1.0, 1.2, 2.3 and 3.2, for exposures of 0, > 0–< 20.6, 20.6–< 60.4 and ≥ 60.4 ppm–years) and leukaemia, and between exposure to 1,3-butadiene and leukaemia. For both of the exposures, a statistically significant relative risk was obtained for the highest cumulative exposure category (for styrene: relative risk, 3.2; 95% CI, 1.2–8.8; 18 deaths; for 1,3-butadiene: relative risk, 3.8; 95% CI, 1.6–9.1; 17 deaths). The exposure–response relationship for DMDTC and leukaemia did not increase monotonically, but a significantly increased relative risk was observed for each of the exposed groups. In models that included all three exposures, the exposure–response

[1] The DMDTC exposure estimation procedure yielded: (1) an estimate of the concentration of DMDTC in the solution wetting the skin of the exposed worker (in mg/cm^3); (2) an estimate of the skin surface exposed (in cm^2); and (3) an estimate of the frequency and duration of exposure. The exposure intensity unit was (mg/cm^3) × (cm^2) = mg/cm.

relationship for styrene became negative. The exposure–response relationship for 1,3-butadiene was weakly positive, and the exposure–response relationship for DMDTC remained irregular. A positive exposure–response model for styrene persisted in a model that included 1,3-butadiene (and not DMDTC). [The Working Group noted the strong correlation between exposure to styrene and 1,3-butadiene and considered this a major obstacle to assessing risks due to styrene *per se*. The Working Group noted that levels of exposure to styrene in this industry were considerably lower than in studies within reinforced plastics industries. The Working Group questioned whether there was sufficient justification for controlling for DMDTC in the analysis since there are no epidemiological or toxicological data suggesting that DMDTC is a potential carcinogen, although it does have toxic effects on haematopoietic tissues in rodents. The results from a previous analysis of this study, based on somewhat different subject pools, somewhat different diagnostic criteria and an earlier exposure assessment (Macaluso *et al*., 1996), were slightly different from those reported by Delzel *et al*. (2001). The Working Group judged the more recent results to be the most informative.]

2.2.4 *Other cohort studies*

In addition to the studies on occupational exposures, a study was conducted on lymphatic and haematopoietic cancers among students attending a high school in Texas adjacent to a styrene–butadiene facility (Loughlin *et al.*, 1999). A cohort of 15 403 students attending this school from 1963–64 to 1992–93 was identified. Altogether, 338 deaths were identified in the 310 254 person–years in the follow-up period from 1963 up to the end of 1995 (SMR, 0.84; 95% CI, 0.74–0.95 among men; SMR, 0.89; 95% CI, 0.73–1.1 among women). There were 44 cancer deaths (SMR, 1.2; 95% CI, 0.83–1.7 among men; SMR, 0.52; 95% CI, 0.28–0.88 among women); 14 deaths were from any lymphatic or haematopoietic cancer [SMR, 1.2; 95% CI, 0.66–2.0 for both genders combined] and seven from leukaemia or aleukaemia (SMR, 1.8; 95% CI, 0.67–4.0 among men; SMR, 0.45; 95% CI, 0.01–2.5 among women; [SMR, 1.3; 95% CI, 0.51–2.6] for both genders combined). The only cause of death with a statistically significant excess was benign neoplasms (six deaths; [SMR, 4.2; 95% CI, 1.5–9.1] for both genders combined; SMR, 6.3; 95% CI, 2.0–15; five deaths among men; SMR, 1.6; 95% CI, 0.04–8.7; one death among women). All six who died of benign neoplasms had brain tumours. [It was not explicitly mentioned how many subjects (men or women) were lost to follow-up. Potential exposures to styrene or other chemical agents were not described.]

A programme of biomonitoring for styrene was undertaken by the Finnish Institute of Occupational Health among workers who were exposed to styrene from 1973. Mandelic acid was used as a biomarker of styrene exposure. Anttila *et al.* (1998) carried out a cancer follow-up study among 2580 subjects who were tested between 1973 and 1983. The overall mandelic acid level was 2.3 mmol/L (range, 0–47 mmol/L urine) during the monitoring period. Altogether 34 288 person–years were accrued in the follow-up from 1973 up to the end of 1992. In the styrene-exposed cohort, 5549 urine

mandelic acid samples were collected; for 84 samples (1.5%), the personal identifier could not be traced. There was no loss to follow-up among those whose personal identifier had been traced successfully [the number of those who migrated was not mentioned.] There were 48 cases of cancer observed (SIR, 0.80; 95% CI, 0.59–1.1); six cases of rectal cancer (SIR, 3.1; 95% CI, 1.1–6.8), three cases of pancreatic cancer (SIR, 1.7; 95% CI, 0.34–4.9), six cases of nervous system cancer (SIR, 1.6; 95% CI, 0.59–3.5) and two cases of lymphatic and haematopoietic cancer (SIR, 0.39; 95% CI, 0.05–1.4; both of the cases had Hodgkin lymphoma). There was no evidence of an exposure–response relationship for any cancer site when mean lifetime mandelic acid level was used as a surrogate for exposure to styrene. There were no cases of pancreatic cancer within this highest exposure category (0.8 cases expected). [The Working Group noted that the relatively small size and low exposures of this cohort may have limited its power to detect any association with lymphatic and haematopoietic neoplasms.]

2.3 Case–control studies

Flodin *et al*. (1986) conducted a matched case–control study of 59 cases of acute myeloblastic leukaemia and 354 controls in Sweden to assess potential risk factors, which included radiation, medications and various occupational exposures. Cases were aged 20–70 years and were identified at hospitals in Sweden between 1977 and 1982. Two series of controls were drawn from a population register: one was matched to cases for sex, age (within five years) and location, and the other was a random population sample. Information on exposure was obtained through a questionnaire mailed to subjects. Of eight occupational exposures examined, styrene was reported by three cases and one control, leading to an estimated standardized odds ratio of 19 (95% CI, 1.9–357). [The Working Group noted the small numbers and the self-reported nature of the exposure estimates, which may be problematic for styrene.]

A population-based case–control study of cancer comprised 3730 histologically confirmed male cases of cancer at 15 major sites (including non-Hodgkin lymphoma and Hodgkin lymphoma) newly diagnosed between 1979 and 1986 in Montreal, Canada, aged 35–70 and ascertained in 19 major hospitals, as well as 533 population controls (Siemiatycki, 1991; Gérin *et al.*, 1998; Dumas *et al.*, 2000). The exposure of each subject to 293 occupational agents was assessed by a group of chemists on the basis of jobs held, and cases of cancer at each site were compared with those in the rest of the study population, after adjustment for age, ethnic group, family income, alcohol drinking and tobacco smoking. Two per cent of the subjects were classified as ever having been exposed to styrene. A synthetic form of cumulative exposures at low, medium or high level was also computed, based on the sum product of duration, frequency and concentration of exposure. The odds ratio for rectal cancer for any exposure to styrene was 1.7 (95% CI, 0.7–4.5; six exposed cases) (Siemiatycki, 1991; Dumas *et al.*, 2000). A significant increase in the risk for cancer of the rectum was seen for medium to high exposure level (odds ratio, 5.1; 95% CI, 1.4–19; five exposed cases in single-exposure model; odds

ratio, 4.4; 95% CI, 1.1–17 in multiple-exposure models adjusting additionally for some other solvents). In the single-exposure models, the odds ratios for exposure to styrene were: 1.2 (95% CI, 0.6–2.5, 11 exposed cases for any exposure) for colon cancer, 0.3 (95% CI, 0.0–2.6; one exposed case for any exposure) for pancreatic cancer, 0.9 (95% CI, 0.2–3.3; five exposed cases for medium to high exposure) for lung cancer, 2.0 (95% CI, 0.8–4.8; eight cases for any exposure) for non-Hodgkin lymphoma and 2.4 (95% CI, 0.5–12; two exposed cases for any exposure) for Hodgkin lymphoma (Gérin *et al.*, 1998). [The Working Group noted that the exposure levels of the exposed subjects were probably lower than those in the cohort studies.]

3. Studies of Cancer in Experimental Animals

Six studies in rats and four studies in mice by oral and inhalation routes of exposure to styrene were presented in the previous monograph (IARC, 1994a). These are included here in summary form together with reviews of new inhalation studies in rats and mice.

Data on the carcinogenicity of styrene 7,8-oxide in experimental animals were evaluated as *sufficient evidence* in the same volume (IARC, 1994b).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, received daily administrations of 150 or 300 mg/kg bw styrene (purity, 99.7%) in corn oil by gavage on five days per week, for 78 weeks, and the animals were killed after a further 13 weeks. Control groups of 20 male and 20 female mice received corn oil alone. The incidence of bronchiolo-alveolar carcinomas in males was 0/20, 3/44 and 5/43, while the incidence of adenomas and carcinomas combined was 0/20, 6/44 and 9/43 ($p = 0.024$) for doses of 0, 150 and 300 mg/kg, respectively. There were no bronchiolo-alveolar carcinomas in female mice. The incidence of bronchiolo-alveolar adenomas in females was 0/20, 1/43 and 3/43, respectively (National Cancer Institute, 1979a). [The Working Group noted the small number of control animals and that the incidence of both adenomas and carcinomas combined was within the historical control ranges.]

Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, received a commercial mixture of β-nitrostyrene ((2-nitroethenyl)benzene) (30% β-nitrostyrene, 70% styrene) in corn oil; the doses of styrene were 204 or 408 mg/kg bw, administered by gavage, on three days per week, for 78 weeks. Animals were killed after a further 14 weeks. Control groups of 20 male and 20 female mice received corn oil alone. There were no increases in the incidence of any tumours (National Cancer Institute, 1979b). [The Working Group noted that a mixture of styrene with β-nitrostyrene was tested.]

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, received daily doses of 1000 or 2000 mg/kg bw styrene (purity, 99.7%) in corn oil by gavage on five days per week, for 78 weeks; survivors were held until 105 weeks. Control groups of 20 male and 20 female rats received corn oil alone. Due to increased treatment-related mortality in the 2000-mg/kg bw group, additional groups of males and females receiving 0 and 500 mg/kg bw styrene per day in corn oil by gavage for 103 weeks were included. No treatment-related increase in the incidence of any type of tumour was observed (National Cancer Institute, 1979a).

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, received commercial β-nitrostyrene (30% β-nitrostyrene, 70% styrene) in corn oil by gavage on three days per week, for 79 weeks, at styrene doses of 175 (females), 350 (males and females) and 750 (males) mg/kg bw; survivors were observed for an additional 29 weeks. No treatment-related increase in the incidence of any type of tumour was observed (National Cancer Institute, 1979b).

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks of age, were administered 0, 50 or 250 mg/kg bw styrene (99.8% pure) in olive oil by gavage on 4–5 days per week for 52 weeks. Survivors were held until death. In males, there was no increase in tumour incidence. In females, there was a decreased incidence of malignant and malignant plus benign mammary tumours at 250 mg/kg bw, which the authors attributed to decreased survival (Conti *et al*., 1988). [The Working Group noted the limited reporting of this study and the short duration of treatment.]

Groups of 50 male and 70 female Charles River COBS (SD) BR rats, seven weeks of age, were administered 125 or 250 ppm (mg/L) styrene (> 98.9% pure) daily in the drinking-water for two years. Groups of 76 male and 106 female rats received drinking-water alone. Dosing was limited by the solubility of styrene in water. Styrene intake was calculated from the concentration, water consumption and body weight as being 7.7 and 14 mg/kg bw per day in males and 12 and 21 mg/kg bw per day in females. No treatment-related increase in the incidence of any type of tumour was observed (Beliles *et al*., 1985).

3.2 Prenatal exposure followed by postnatal oral administration

3.2.1 *Mouse*

A group of 29 pregnant O20 female mice, a strain very susceptible to the formation of lung tumours, was administered 1350 mg/kg bw styrene (99% pure) by gavage in olive oil on day 17 of gestation. Pups (45 male, 39 female) were then given a high dose of 1350 mg/kg bw styrene by gavage weekly following weaning. Treatment was terminated after 16 weekly doses due to toxicity and mortality. All mice were held until spontaneous death, euthanasia due to moribund condition, or until 120 weeks. Lung tumour incidence was increased in both males (8/19 controls versus 20/23 treated) and females (14/21

controls versus 32/32 treated). No increase in tumours at other sites was observed (Ponomarkov & Tomatis, 1978). [The Working Group noted the high treatment-related toxicity and mortality early in the study.]

Using a similar study design, a group of 15 pregnant C57BL mice received 300 mg/kg bw styrene (99% pure) by gavage in olive oil on day 17 of gestation. Twenty-seven male and 27 female offspring received weekly gavage doses of 300 mg/kg bw styrene from weaning for 120 weeks. There was no increase in the incidence of treatment-related tumours (Ponomarkov & Tomatis, 1978).

3.2.2 *Rat*

A group of 21 pregnant BD IV rats was given 1350 mg/kg bw styrene (99.8% pure) by gavage in corn oil on day 17 of gestation; 10 pregnant rats received corn oil only. Beginning at weaning, 73 male and 71 female offspring received 500 mg/kg bw styrene once per week for 120 weeks; 36 male and 39 female control offspring received corn oil once a week for 120 weeks. There was no increase in any tumour incidence in the styrene-treated rats (Ponomarkov & Tomatis, 1978).

3.3 Inhalation exposure

3.3.1 *Mouse*

Groups of 50 male and 50 female mice CD-1 mice, approximately four weeks of age, were exposed by whole-body inhalation to 0, 20, 40, 80 or 160 ppm [85, 170, 341 or 682 mg/m^3] styrene vapour (> 99.5% pure) for 6 h per day, five days per week, for 104 (males) and 98 weeks (females). Due to mortality in control females (23/50 mice), the surviving females were killed six weeks earlier than originally scheduled; all four treated groups had greater survival than the controls (32, 33, 34 and 35/50 for 20, 40, 80 and 160 ppm dose groups, respectively). Other groups of 10 males and 10 females from each exposure level were killed after 52 and 78 weeks. Male and female mice exposed to 80 and 160 ppm had decreased body weights over the course of the study. Statistically significantly increased incidences of bronchiolo-alveolar adenomas were seen in males exposed to 40, 80 or 160 ppm for 24 months (control, 15/50; 20 ppm, 21/50; 40 ppm, 35/50 [$p < 0.05$]; 80 ppm, 30/50 [$p < 0.05$]; 160 ppm, 33/50 [$p < 0.05$]) with no dose–response relationship, but the incidences of bronchiolo-alveolar carcinomas were not increased (4, 5, 3, 6 and 7/50 for 0, 20, 40, 80 and 160 ppm). In females, the incidences of bronchiolo-alveolar adenomas in the groups exposed to 20, 40, and 160 ppm, but not 80 ppm, for 22.5 months were statistically significantly increased (control, 6/50; 20 ppm, 16/50 [$p < 0.05$]; 40 ppm, 16/50 [$p < 0.05$]; 80 ppm, 11/50; 160 ppm, 24/50 [$p < 0.05$]). In females, the incidences of bronchiolo-alveolar carcinoma were 0, 0, 2, 0 and 7/50 ($p < 0.05$) for 0, 20, 40, 80 and 160 ppm. The 14% incidence at 160 ppm was slightly higher than the historical control range of 0–4% for the investigating laboratory (five oral studies) and 0–13.5% for the breeder's database (nine oral studies). No increase in the

incidence of lung tumours was seen in males or females at 12 or 18 months. The increase seen after 24 months was largely in small tumours, as demonstrated by a decreased average tumour size compared with controls. The fact that increased tumour incidences was seen only later than 18 months and the small size of these tumours indicated that these were late-developing tumours. No difference in tumour morphology between control and treated mice was seen. Epithelial hyperplasia of the terminal bronchioles extending into the alveolar duct was seen in a dose-related pattern at all interim and terminal necropsies. Hyperplasia was preceded by decreased eosinophilic staining of Clara cells and cellular crowding (Cruzan *et al*., 2001).

3.3.2 *Rat*

Groups of 30 male and 30 female Sprague-Dawley rats, 12 weeks of age, were exposed by inhalation to 0, 25, 50, 100, 200 or 300 ppm [106, 213, 430, 850 or 1280 mg/m^3] styrene (99.8% pure) for 4 h per day, five days per week, for 12 months (and then held until death). In females, malignant mammary tumours were diagnosed in 6/60 (10%), 6/30 (20%), 4/30 (13%), 9/30 (30%), 12/30 (40%) and 9/30 (30%) rats inhaling 0, 25, 50, 100, 200 or 300 ppm, respectively. For total mammary tumours, the incidences were 34/60 (57%), 24/30 (80%), 21/30 (70%), 23/30 (77%), 24/30 (80%) and 25/30 (83%) for the respective exposure levels (Conti *et al*., 1988). [The Working Group noted the short duration of treatment, the incomplete reporting of the study and the high incidence of spontaneous mammary tumours in animals of this strain.]

Groups of 60 male and 60 female Charles River CD rats, approximately four weeks of age, were exposed by whole-body inhalation to 0, 50, 200, 500 or 1000 ppm [213, 850, 2130 or 4260 mg/m^3] styrene (> 99.5% pure) for 6 h per day on five days per week for 104 weeks. Females exposed to 500 and 1000 ppm weighed less than controls throughout the study. However, there was a dose-related increase in survival; at termination, survival was 48, 47, 48, 67 and 82% in females exposed to 0, 50, 200, 500 and 1000 ppm, respectively. There were no increased incidences of tumours related to styrene exposure. A dose-dependent decrease in mammary tumours in females was reported. Mammary adenocarcinomas were diagnosed in 20/61 (33%), 13/60 (22%), 9/60 (15%), 5/60 (8%) and 2/60 (3%) female rats exposed to 0, 50, 200, 500 or 1000 ppm styrene, respectively, for two years. A decrease in benign mammary fibroadenomas (including those with epithelial atypia) was seen, the incidence being 27/61 (44%), 22/60 (37%), 18/60 (30%), 21/60 (35%) and 19/60 (32%) for the above exposure levels, respectively (Cruzan *et al*., 1998).

3.4 Intraperitoneal administration

3.4.1 *Mouse*

In a screening assay based on multiplicity and incidence of lung tumours in a highly susceptible strain of mice (A/J), administration of 200 μmol (~100 mg/kg bw) styrene by intraperitoneal injection three times per week for 20 doses, followed by observation for 20 weeks, produced no increase in lung adenoma incidence in the styrene-treated mice compared with controls (Brunnemann *et al*., 1992).

3.4.2 *Rat*

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks of age, received four intraperitoneal injections of 50 mg styrene (99.8% pure) per animal in olive oil at two-month intervals. Control groups received olive oil alone. The study was terminated when the last rat died [duration unspecified]. There was no increase in tumour incidence (Conti *et al*., 1988). [The Working Group noted the incomplete reporting of data, the short duration of treatment and the low total dose.]

3.5 Subcutaneous administration

Rat: Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks of age, received a single subcutaneous injection of 50 mg styrene (> 99% pure) per animal in olive oil. Control groups received olive oil alone. The study was terminated when the last rat died [duration unspecified]. There was no increase in tumour incidence (Conti *et al*., 1988). [The Working Group noted the incomplete reporting of data and the single low-dose treatment.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

Studies on the pharmacokinetics and metabolism of styrene were evaluated in 1994 as part of a previous IARC monograph (IARC, 1994a), which may be consulted for more details on the earlier studies.

4.1.1 *Humans*

(*a*) *Absorption*

As noted previously (IARC, 1994a), the pulmonary retention of styrene is 60–70% of the inhaled dose based on studies in both volunteers and workers (Stewart *et al*.,

1968; Engström *et al*., 1978a,b; Ramsey *et al*., 1980; Wigaeus *et al*., 1983, 1984; Pezzagno *et al*., 1985; Wieczorek & Piotrowski, 1985; Löf *et al*., 1986a,b). This has been confirmed by more recent studies (Johanson *et al*., 2000; Wenker *et al*., 2001a,b). Wrangskog *et al*. (1996) developed a simple one-compartment model for estimation of styrene uptake based on measurements of urinary excretion of mandelic and phenylglyoxylic acids.

In human volunteers exposed by placing one hand in liquid styrene for 10–30 min, absorption was low, averaging 1 μg/cm^2/min (Berode *et al*., 1985). Limasset *et al*. (1999) carried out a field study comparing urinary excretion of metabolites of styrene in four groups of workers who performed the same task but wore different protective equipment (see Section 1.4.1(*d*)), and concluded that percutaneous absorption of styrene was not an important contribution to the body burden.

(*b*) *Distribution*

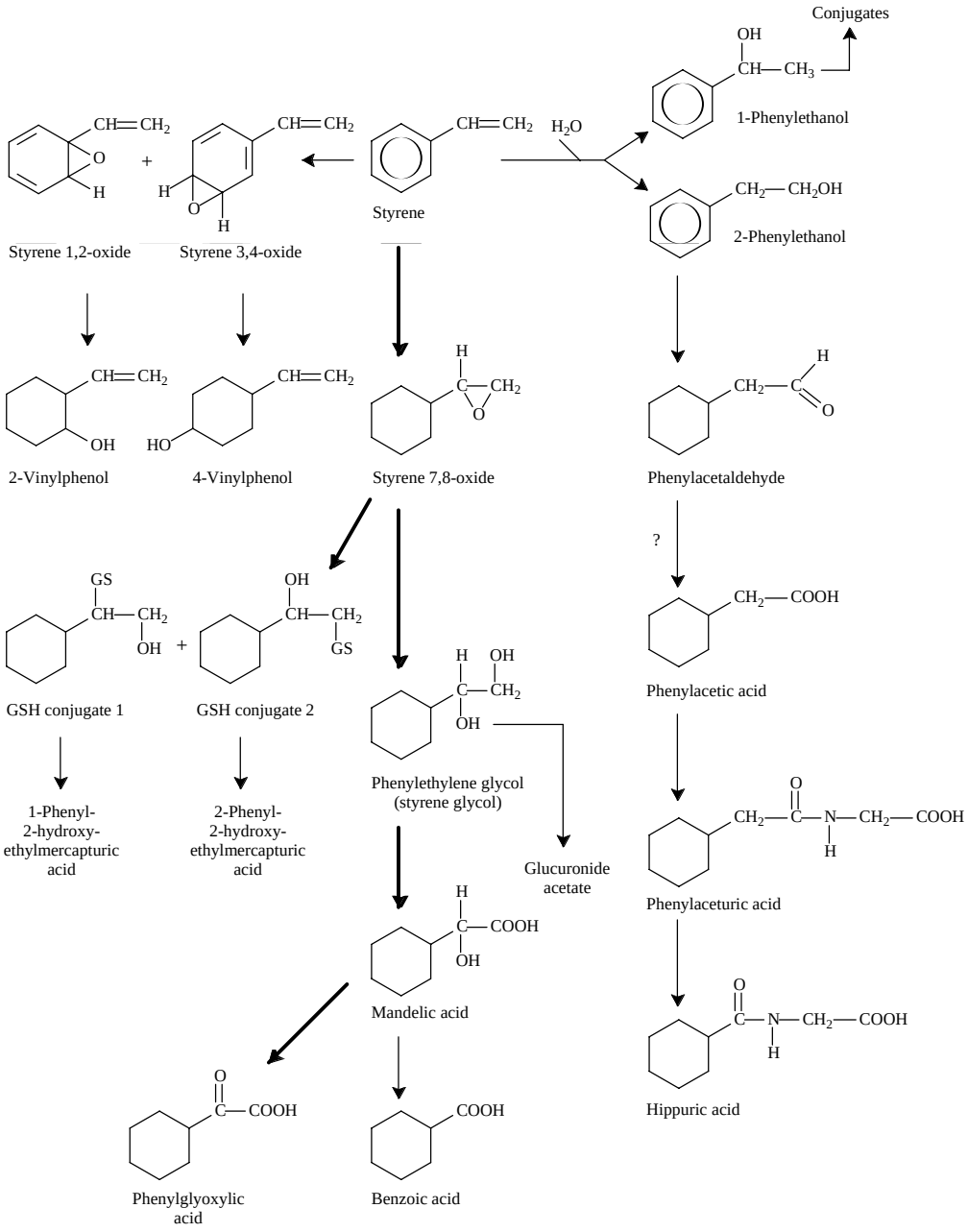
Several studies have suggested that styrene accumulates in subcutaneous fat (Wolff *et al*., 1977; Engström *et al*., 1978a,b). However, based on measurement of urinary metabolites, workers exposed to 37 ppm (160 mg/m^3) styrene showed no accumulation during the working week (Pekari *et al*., 1993). As noted below, pharmacokinetic analysis of the disposition of styrene supports this conclusion.

(*c*) *Metabolism*

The metabolic pathways for styrene are shown in Figure 1. Styrene is primarily metabolized to styrene 7,8-oxide by cytochrome P450 (CYP) enzymes. The oxide is metabolized by epoxide hydrolase to phenylethylene glycol and then to mandelic, phenylglyoxylic and benzoic acids. Additional routes of metabolism include ring hydroxylation, but this appears to be a minor pathway in humans. Pfäffli *et al*. (1981) identified small amounts of 4-vinylphenol in the urine of workers exposed to styrene (less than 1% of the amount of mandelic acid excreted by these workers). Another pathway is conversion of styrene to 1- and 2-phenylethanol, which is further metabolized to phenylacetaldehyde, phenylacetic acid, phenylaceturic acid and hippuric acid. Styrene 7,8-oxide may also be metabolized by conjugation with glutathione to form mercapturic acids. This appears to be a minor pathway in humans, amounting to less than 1%. Ghittori *et al*. (1997) evaluated urinary excretion in 22 workers in a reinforced-plastics factory (see Section 1.1.5(*b*)) and identified racemic mercapturates, i.e., the *R*- and *S*-diastereoisomers of *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)cysteine and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)cysteine. Assuming a styrene uptake of 64%, the conversion to mercapturic acids was calculated to be between 0.021 and 0.325% of the dose.

Korn *et al*. (1994) found a linear correlation between concentrations of styrene 7,8-oxide in the blood of workers exposed to styrene (10–73 ppm [43–310 mg/m^3]) and styrene in ambient air and blood. At 20 ppm [85 mg/m^3] styrene, the steady-state level of styrene 7,8-oxide was about 1 μg/L blood.

Figure 1. Main metabolic pathways for styrene



From IARC (1994a). Main pathways are indicated by thick arrows.
GSH, glutathione

Many investigators have used measurements of urinary mandelic and phenylglyoxylic acids as biomarkers of exposure in workers (reviewed by Guillemin & Berode, 1988) (see Section 1.1.5(*b*)). Stereochemical studies have been carried out on these metabolites. Korn *et al*. (1984) found the average ratio of L-mandelic acid to D-mandelic acid to be 1.62 in 11 male styrene-exposed workers and 1.44 in six female workers. In 20 male volunteers exposed to styrene (360 mg/m^3) for 1 h while performing 50-W physical exercise, the average pulmonary retention was 62%, with mandelic acid and phenylglyoxylic acid accounting for 58% of the absorbed styrene. The maximum concentration and area-under-the-curve for the free *R*-styrene glycol in blood were 1.3 and 1.7 times higher, respectively, than those of the *S*-styrene glycol. Cumulative excretion and renal clearance of conjugated styrene glycol were three and four times higher for *S*-styrene glycol than for *R*-styrene glycol, respectively (Wenker *et al*., 2001a).

Studies in humans have attempted to assess interactions of chemicals that might alter styrene metabolism by comparing data on metabolite concentrations in urine of workers exposed to mixtures with historical information on workers exposed to styrene alone. Kawai *et al*. (1992) measured urinary metabolites of styrene in 34 male workers engaged in the production of plastic buttons and exposed to a mixture of styrene (mean concentration, 7 ppm [30 mg/m^3]), toluene and methanol. Styrene metabolism was similar to that reported previously in populations exposed to styrene alone (Ikeda *et al.*, 1982). This conclusion was supported by a subsequent study of 39 male workers co-exposed to methanol and 12.4 ppm [53 mg/m^3] styrene (Kawai *et al*., 1995). Apostoli *et al*. (1998) examined the urinary excretion of mandelic and phenylglyoxylic acids in 50 workers involved in the production of polyester buttons who were exposed to styrene (16–439 mg/m^3) and acetone (15–700 mg/m^3). In groups with different levels of acetone co-exposure, the acetone had no effect on excretion of these metabolites. Similarly, De Rosa *et al*. (1993) monitored mandelic acid excretion in 22 workers exposed to styrene (22–522 mg/m^3) and acetone (40–1581 mg/m^3) and found no effect due to the co-exposure to acetone. Cerný *et al*. (1990) examined the metabolism of styrene (exposure concentration, 420 mg/m^3) for 8 h with and without co-administration of ethanol (four doses of 14 g given at 2-h intervals) in five volunteers and found that this acute administration decreased the excretion of mandelic and phenylglyoxylic acids. To examine whether exposure to styrene would increase styrene metabolism in subsequent exposures, Wang *et al*. (1996) used the data of Wigaeus *et al*. (1983) and Löf *et al*. (1986a) from a study in which two groups of subjects, one previously exposed to styrene and one not, were exposed to 80 ppm [341 mg/m^3] styrene for 2 h, and estimated the metabolic rate constant based on a three-compartment pharmacokinetic model. No effect on styrene metabolism was found, but the number of subjects in this study (six or seven per group) was limited.

Johanson *et al*. (2000) exposed four volunteers for 2 h to 50 ppm [213 mg/m^3] [$^{13}C_8$]styrene vapour via a face mask while they performed light physical exercise. Two hours after exposure had ended, the concentration of styrene 7,8-oxide in blood reached

a maximum and averaged 6.7 nM. There was no evidence of glutathione conjugation or ring epoxidation.

Individual differences in the metabolic response to styrene were studied in 20 male volunteers exposed on separate occasions to 104 and 360 mg/m^3 styrene for 1 h while performing 50-W exercise. Urinary mandelic and phenylglyoxylic acids were measured to make correlations with individual metabolic capacities determined with enzyme-specific substrates for CYP2E1, CYP1A2 and CYP2D6. No correlation was found among individuals between the blood clearance of styrene and the metabolic capacity. Based on the high apparent blood clearance of styrene (1.4 L/min), one explanation proposed by the authors is that styrene metabolism is limited by the blood flow to the liver, which has a similar value (Wenker *et al*., 2001b). Symanski *et al*. (2001) examined inter- and intra-individual differences in urinary concentrations of mandelic and phenylglyoxylic acids based on 1714 measurements in 331 workers over the period 1985–99. Interindividual differences were greater for post-shift urine samples than for pre-shift samples.

Hallier *et al*. (1995) measured *R*- and *S*-mandelic acids in the urine of 20 male workers exposed to 29–41 ppm styrene [124–175 mg/m^3]. The ratio of the *R*- to *S*-enantiomers of mandelic acid ranged from 0.7 to 2.2. This variation could not be explained by differences in exposure levels and was attributed to interindividual differences in styrene metabolism, probably related to enzyme polymorphisms.

The role of specific CYP isozymes in the metabolism of styrene has been examined. Nakajima *et al*. (1994a), using 12 different purified human isozymes, determined that CYP2B6 and CYP2E1 were the most active in human liver microsomes in forming styrene glycol, and CYP2F1 was the most active in lung microsomes. Guengerich *et al*. (1991) also demonstrated the significance of CYP2E1 in styrene metabolism, using diethyldithiocarbamate as a specific inhibitor of CYP2E1 and correlations with chlorzoxazone 6-hydroxylation — an indicator of CYP2E1 activity — in human liver microsomes. Using antibodies against specific human CYP isozymes and by comparing rates of styrene glycol formation by microsomes isolated from human livers, Kim *et al*. (1997) identified CYP2E1 and CYP2C8 as being the most important metabolic enzymes at low styrene concentration (0.085 mM), while CYP2B6 and CYP2C8 were most prominent at a high concentration of styrene (1.8 mM).

Wenker *et al*. (2000) examined the stereospecificity and interindividual variation in microsomal epoxide hydrolase activity in 20 human liver samples. V_{max}, K_m and V_{max}/K_m values for the substrates *R*- and *S*-styrene 7,8-oxides varied three- to five-fold between livers. The rate of hydrolysis of *S*-styrene 7,8-oxide was approximately five times slower than that of *R*-styrene 7,8-oxide, although the K_m was six times higher for *S*-styrene 7,8-oxide. In a further report (Wenker *et al*., 2001c), an eightfold variation in V_{max} was found for styrene metabolism by 20 human liver samples (0.39–3.2 nmol/mg protein/min), and, although CYP2E1 was found to be the most important cytochrome P450 isoform, there was no correlation between the enzymic activity (V_{max} or K_m) and genetic polymorphisms of this enzyme.

Haufroid *et al*. (2001) examined possible influences of genetic polymorphisms in metabolic enzymes on the metabolism of styrene (average concentration, 18.2 ppm [77.5 mg/m^3]) in 30 workers from a glass fibre-reinforced plastics factory. The presence of the rare *CYP2E1*1B* allele was associated with increased excretion of urinary mandelic and phenylglyoxylic acids as well as mercapturic acid metabolites. Individuals deficient in glutathione *S*-transferase (GST) M1 excreted less than one-half the amount of mercapturic acids, a minor pathway in humans, compared with *GSTM1*– proficient individuals.

De Palma *et al*. (2001) examined polymorphisms in GSTM1-1 and T1-1 and microsomal epoxide hydrolase in 56 styrene-exposed workers (geometric mean exposure, 157 mg/m^3, time-weighted average over 8 h). GSTM1-1 was identified as the primary isozyme for conjugation of glutathione with styrene 7,8-oxide. Workers who were positive for GSTM1-1 excreted 5–6 times more phenylhydroxyethylmercapturic acids than did individuals who were GSTM1-1-deficient. No association was found between microsomal epoxide hydrolase polymorphisms and excretion of the mercapturic acids.

(*d*) *Excretion*

Only small amounts of styrene (0.7–4.4%) are exhaled unchanged (see IARC, 1994a for details). This has been confirmed in more recent studies, in which 0.7–2.2% of the amount of inhaled styrene was found unchanged in the exhaled breath of four subjects exposed to 50 ppm [213 mg/m^3] styrene for 2 h (Johanson *et al.*, 2000). Small amounts of styrene are also excreted unmetabolized in the urine (Pezzagno *et al*., 1985; Gobba *et al*. 1993). Ramsey *et al*. (1980) examined the pharmacokinetics of inhaled styrene (80 ppm [341 mg/m^3]) in four human volunteers and calculated half-life values of 0.6 and 13.0 h for the two phases of elimination.

Brugnone *et al*. (1993) measured styrene concentrations in blood of 76 exposed workers at the end of the work shift and the morning after, and reported a half-life of elimination of 3.9 h.

4.1.2 *Experimental systems*

(*a*) *Absorption*

McDougal *et al*. (1990) compared the dermal absorption of vapours from several organic chemicals in male Fischer 344 rats. The animals breathed fresh air through a latex mask. For styrene (3000 ppm [12 800 mg/m^3] for 4 h), a maximal blood concentration of about 10 µg/mL and a permeability constant of 1.753 cm/h were reported. In a mixed exposure situation (inhalation and skin absorption), the skin uptake was estimated to account for 9.4% of the total uptake in these rats. Morgan *et al*. (1991) examined the dermal absorption of styrene in Fischer 344 rats. When administered neat (2 mL of styrene in a sealed dermal cell), the peak blood level of styrene, reached after 1 h, was 5.3 µg/mL. Absorption decreased when the styrene was diluted with water.

(*b*) *Distribution*

An early study in which [^{14}C]styrene labelled at the β-carbon atom was administered subcutaneously to Wistar rats (0.10 mL of a 20% solution to 100–125-g rats [20 mg per animal]) indicated that styrene was distributed rapidly (< 1 h) to tissues and that excretion of the radioactivity was primarily via the urine, with only a small fraction (2–3%) exhaled unchanged by the lung and approximately 12% appearing as $^{14}CO_2$ during 24 h after dosing (Danishefsky & Willhite, 1954).

Teramoto and Horiguchi (1979) exposed rats [strain unspecified] to 500 and 1000 ppm [2130 and 4260 mg/m^3] styrene by inhalation for 4 h and found the highest concentration of styrene in adipose tissue. The biological half-life in this tissue was 6.3 h, as opposed to 2.0–2.4 h in blood, liver, kidney, spleen, muscle and brain. No accumulation of styrene was found when rats were exposed to 700 ppm [2980 mg/m^3] for 4 h per day for five days. Withey and Collins (1979) exposed Wistar rats to styrene by inhalation (50–2000 ppm [213–8520 mg/m^3] for 5 h) and found a proportionally increasing level of styrene uptake into blood. Tissue distribution was concentration-dependent, and the concentration of styrene in the perirenal fat was higher than in any other tissue. This is probably a consequence of the high oil:blood partition coefficient of styrene (130; see Van Rees, 1974). When [^{14}C]styrene was administered orally to rats (20 mg/kg), the highest levels of radioactivity were found in the kidney followed by the liver and pancreas. Ninety per cent of the dose appeared in the urine within 24 h after dosing (Plotnick & Weigel, 1979). Löf *et al*. (1983, 1984) studied the effect of time and dose on styrene distribution and metabolism following intraperitoneal administration of styrene (1.1–4.9 mmol/kg bw) to NMRI mice. The highest concentrations of styrene were measured in adipose tissue, pancreas, liver and brain. Styrene 7,8-oxide concentrations were highest in kidney and subcutaneous adipose tissue and lowest in lung. The highest concentrations of styrene glycol were found in kidney, liver, blood and lungs. Male Wistar rats given styrene (100 mg/kg) by intubation excreted both *R*- and *S*-mandelic acids, with a preference towards excretion of the *R*-form (Drummond *et al.*, 1989).

The uptake of styrene by the upper respiratory tract of the CD-1 mouse and Sprague-Dawley rat has been determined using a surgically isolated upper respiratory tract preparation (Morris, 2000). In rats, steady-state uptake efficiency of styrene decreased with increasing exposure concentration, with 24% and 10% efficiency at 5 and 200 ppm [21 and 852 mg/m^3], respectively. In mice, the uptake efficiency — which did not maintain a steady state but declined during exposure — averaged between 42% and 10% at 5–200 ppm. Treatment with metyrapone, an inhibitor of cytochrome P450, abolished the concentration-dependence of the uptake efficiency in both species, providing evidence that inhaled styrene is metabolized by nasal tissues and that this determines the concentration-dependence.

(*c*) *Metabolism*

Watabe *et al*. (1978) clearly demonstrated the NADPH-dependent conversion of styrene to styrene 7,8-oxide in liver microsomes from male Wistar-King rats, with further conversion to styrene glycol by epoxide hydrolase.

The role of glutathione conjugation in the metabolism of styrene was recognized by Seutter-Berlage *et al*. (1978), who characterized urinary mercapturic acids amounting to approximately 11% of an intraperitoneal dose of styrene (250 mg/kg bw) in Wistar rats. The compounds were identified as *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)cysteine, *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)cysteine and *N*-acetyl-*S*-(phenacyl)cysteine, which were present in a 65:34:1 ratio (Seutter-Berlage *et al.*, 1978; Delbressine *et al*., 1981). Watabe *et al*. (1982) administered styrene, racemic styrene 7,8-oxide, *R*-styrene 7,8-oxide and *S*-styrene 7,8-oxide (all at 2 mmol/kg bw) to male Wistar rats and found the two major metabolites mentioned above. For the *R*-enantiomer, the rate of metabolism to mercapturic acid was 2.5 times higher than for the *S*-enantiomer. Nakatsu *et al*. (1983) similarly identified these metabolites in the urine of male Sprague-Dawley rats after intraperitoneal administration of styrene 7,8-oxide (100 mg/kg bw). Truchon *et al*. (1990) exposed Sprague-Dawley rats to styrene by inhalation (25–200 ppm [106–852 mg/m^3], 6 h per day, five days per week for four weeks) and found dose-dependent excretion of *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)cysteine and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)cysteine in urine. Recent attention has focused on stereochemical aspects of the formation of these mercapturic acids. Coccini *et al.* (1996) exposed Sprague-Dawley rats subchronically to styrene (300 ppm [1280 mg/m^3], 6 h per day, five days per week for two weeks). Urine was collected during the last 6 h of exposure. Approximately 6.5% of the dose was recovered as *N*-acetyl-*R*-(1-phenyl-2-hydroxyethyl)cysteine, 19.5% as *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)cysteine and 10.3% as *N*-acetyl-*S*- or *R*-(2-phenyl-2-hydroxyethyl)cysteine. Linhart *et al*. (1998) dosed Wistar rats intraperitoneally with *R*-, *S*- and racemic styrene 7,8-oxide (150 mg/kg bw) and found that the regioselectivity was similar for all three treatments, yielding a 2:1 mixture of *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)cysteine and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)cysteine, although the conversion to the mercapturic acids was higher with *R*-styrene 7,8-oxide (28% of the dose) than with the *S*-isomer (19%). In male B6C3F$_1$ mice given an intraperitoneal injection of 400 mg/kg bw styrene, the two major urinary metabolites were *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine and *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl) cysteine, together comprising 12.5% of the dose (Linhart *et al*., 2000).

Several studies have focused on the influence of inducers and inhibitors of xenobiotic metabolism on the biotransformation of styrene. Treatment of female Wistar rats with sodium phenobarbital (37.5 mg/kg bw injected intraperitoneally, twice daily for four days) increased the metabolism of styrene (given by intraperitoneal injection of 455 mg/kg bw on the fifth day), as determined by measurements of urinary metabolites (Ohtsuji & Ikeda, 1971). Sato and Nakajima (1985) found that treatment of male Wistar

rats with phenobarbital (oral dose of 80 mg/kg bw daily for three days) enhanced the hepatic microsomal metabolism of styrene approximately six-fold. Elovaara *et al.* (1991) compared the influence of acetone, phenobarbital and 3-methylcholanthrene on the urinary metabolites of male Han/Wistar rats exposed to styrene by inhalation (2100 mg/m^3 for 24 h) and found increases of 30–50% in the excretion of phenylglyoxylic acid and mandelic acid in the rats treated with acetone (1% in drinking-water for one week) but not in the other groups. Co-administration by intraperitoneal injection of toluene (217 mg/kg bw) suppressed the metabolism of styrene (228 mg/kg bw) in Wistar rats (Ikeda *et al*., 1972). Co-administration of ethanol (2 mM) decreased the uptake and metabolism of styrene (given as a 28.8-µM solution) in a perfused rat liver system in fed animals and increased these parameters in fasted animals. This was associated with increased levels of NADPH due to oxidation of ethanol (Sripaung *et al*., 1995).

Salmona *et al*. (1976) compared formation of styrene 7,8-oxide and its further transformation to styrene glycol by epoxide hydrolase in microsomal preparations from different tissues from CD rats and found mono-oxygenase and epoxide hydrolase activity in liver, lung and kidney, but not in heart, spleen and brain, with the highest activities in male rat liver.

When apparent hepatic V_{max} values for the metabolism of styrene to styrene 7,8-oxide by styrene mono-oxygenase were compared among species, the order was Dunkin Hartley guinea-pig > New Zealand rabbit > Swiss mouse > Sprague-Dawley rat (Belvedere *et al*., 1977). However, the order for the metabolism of styrene 7,8-oxide to styrene glycol by epoxide hydrolase was rat > rabbit > guinea-pig >mouse, so that the V_{max} ratios for epoxide hydrolase *vs* styrene mono-oxygenase were 3.9, 2.4, 1.6 and 1.4 for the rat, rabbit, guinea-pig and mouse, respectively. Watabe *et al*. (1981) reported that liver microsomal preparations from male Wistar rats preferentially formed *S*-styrene 7,8-oxide over *R*-styrene 7,8-oxide (*R*- to *S*-ratio, 0.77). Foureman *et al*. (1989) examined the stereoselectivity of styrene 7,8-oxide formation in the livers of male Sprague-Dawley rats and found an *R*- to *S*- ratio of 0.65. This ratio increased to 0.92 in phenobarbital-treated rats. This *R*- to *S*-ratio contrasts with results indicating preferential formation of *R*-styrene 7,8-oxide in the liver of CD-1 mice (ratio *R*/*S*, 1.2–1.8) (Carlson, 1997a; Hynes *et al*., 1999) and in pulmonary microsomes from rabbits (ratio of 1.6) (Harris *et al*., 1986).

Mendrala *et al*. (1993) compared the kinetics of styrene and styrene 7,8-oxide metabolism in rat, mouse and human livers *in vitro*. K_m values for styrene epoxidation by mono-oxygenase were similar for the three species. The V_{max} values, however, varied from 13 nmol/min/mg protein for B6C3F$_1$ mice, 11 nmol/min/mg protein for Fischer 344 rats, 9.3 nmol/min/mg protein for Sprague-Dawley rats to 2.1 nmol/min/mg protein for humans (average of five donors). V_{max} values for epoxide hydrolase were similar for all three species (14–15 nmol/min/mg protein).

Nakajima *et al*. (1994a) compared the rates of styrene metabolism using microsomal preparations from different sources. At a low substrate concentration (0.085 mM), the rates of hepatic metabolism decreased from mouse (2.43 nmol/mg protein/min) to rat

(1.07 nmol/mg protein/min) to human (0.73 nmol/mg protein/min), whereas at a high styrene concentration (1.85 mM), the rates were in the order of rat (4.21 nmol/mg protein/min) to mouse (2.72 nmol/mg protein/min) to human (1.91 nmol/mg protein/min). Activity in pulmonary microsomes from 38 individuals was much lower (0.006–0.0125 nmol/mg protein/min) than in liver. Carlson *et al*. (2000) also observed low activity for styrene metabolism in microsomal preparations from six human lungs. Styrene 7,8-oxide formation was detectable in only one human sample (0.088 nmol/mg protein/min). [The Working Group noted that enzyme activity measurements based upon analysis of whole organ homogenates do not reflect the substantial differences that exist between cell types, as has been demonstrated by immunohistochemical methods for CYP2E1 and CYP2F2 in lung (Buckpitt *et al*., 1995; Green *et al*., 1997).]

A few studies have described the formation of the ring-hydroxylated product 4-vinylphenol after exposure of rodents to styrene. Bakke and Scheline (1970) reported finding 4-vinylphenol in amounts up to 0.1% of the dose in enzymatically hydrolysed urine of male rats [strain unspecified] dosed orally with styrene (100 mg/kg bw). Similarly, Pantarotto *et al.* (1978) identified small amounts of 4-vinylphenol, 4-hydroxymandelic acid, 4-hydroxybenzoic acid and 4-hydroxyhippuric acid in the urine of male Sprague-Dawley rats administered styrene intraperitoneally. More recently, when rats and mice were exposed by inhalation to [*ring*-U-^{14}C]styrene, Boogaard *et al.* (2000a) reported finding $^{14}CO_2$, suggesting that ring hydroxylation may occur followed by ring opening. The fraction of the dose eliminated as $^{14}CO_2$ varied from 6.4–8.0% of the retained dose of styrene in mice to 2% of the retained dose in rats. Watabe *et al.* (1984) demonstrated the formation of 4-vinylphenol using ^{14}C-labelled styrene and a rat hepatic microsomal preparation fortified with NADPH after addition of a large amount of unlabelled 4-vinylphenol as a trapping agent. They suggested that 4-vinylphenol was formed via the 3,4-oxide but very rapidly metabolized to 4-hydroxystyrene-7,8-glycol. Carlson *et al*. (2001) were unable to confirm 4-vinylphenol formation when styrene was incubated with hepatic and pulmonary microsomal preparations from CD-1 mice and Sprague-Dawley rats. However, considerable 4-vinylphenol-metabolizing activity, as determined by the loss of 4-vinylphenol, was observed in both mouse and rat liver and lung microsomal preparations. This activity was three times higher in mouse liver microsomes than in rat liver microsomes and eight times higher in mouse lung microsomes than in rat lung microsomes, and it was completely absent in the absence of NADPH. Studies with cytochrome P450 inhibitors indicated the involvement of CYP2E1 and CYP2F2.

Several studies have focused on the pharmacokinetics of styrene and some species comparisons. Ramsey and Young (1978) exposed Sprague-Dawley rats to styrene by inhalation at concentrations of 80, 200, 600 and 1200 ppm [341, 852, 2560 and 5110 mg/m^3] for up to 24 h. The increase in styrene concentration in blood with increased exposure concentration was non-linear, indicating saturation of metabolism after about 6 h of exposure. Filser *et al*. (1993) compared male Sprague-Dawley rats and B6C3F$_1$ mice exposed to styrene by inhalation in a closed chamber (550–5000 ppm [2340–21 300 mg/m^3]), by intraperitoneal injection (20–340 mg/kg bw) or by oral

gavage (100–350 mg/kg bw). In both species, the rate of metabolism of the inhaled styrene was concentration-dependent. At exposure concentrations above 300 ppm [1280 mg/m^3], metabolism was progressively limited by metabolic capacity and was saturated at concentrations of approximately 700 ppm [2980 mg/m^3] in rats and 800 ppm [3410 mg/m^3] in mice. Little accumulation occurred at exposures below 300 ppm [1280 mg/m^3] since uptake was rate-limiting. In rats and mice, intraperitoneal injection of styrene followed by analysis of exhaled styrene in the closed chamber resulted in concentration–time curves in agreement with the applied pharmacokinetic model. After oral administration of styrene, the concentration–time curves showed considerable inter-animal variability.

In a chronic inhalation study in Sprague-Dawley rats exposed (whole-body) to styrene vapour at 0, 50, 200, 500 or 1000 ppm [0, 213, 852, 2130 or 4260 mg/m^3] for 6 h per day, five days per week for 104 weeks, blood levels of styrene and styrene 7,8-oxide were measured at the end of a 6-h exposure period during week 95. Styrene 7,8-oxide in blood was undetectable in males or females at 0 or 50 ppm. While the styrene 7,8-oxide concentration in blood continued to increase with higher styrene exposure concentration, some saturation of metabolism was observed (Cruzan *et al*., 1998). In CD-1 mice exposed to styrene vapour at 0, 20, 40, 80 or 160 ppm [0, 85, 170, 341 or 682 mg/m^3] for 98 weeks (females) or 104 weeks (males), blood levels of styrene and styrene 7,8-oxide were measured at the end of a 6-h exposure period during week 74. Styrene 7,8-oxide in blood was undetectable in males and females at 0 and 20 ppm. At higher exposure doses, concentration-dependent increases were observed for styrene and styrene 7,8-oxide in blood, with no evidence for saturation of metabolism (Cruzan *et al*., 2001). Comparing the two studies, blood styrene concentrations in male and female rats reached 2780 ng/mL and 1950 ng/mL at 200 ppm styrene exposure and blood styrene 7,8-oxide concentrations reached 66 ng/mL and 28 ng/mL at this exposure level, respectively, whereas for mice the corresponding values at the highest dose (160 ppm) were 1461 ng/mL and 1743 ng/mL for styrene and 33.5 ng/mL and 20.1 ng/mL for styrene 7,8-oxide in males and females, respectively.

The effect of antibodies to CYP2C11/6, CYP2B1/2, CYP1A1/2 and CYP2E1 on the rates of styrene metabolism was studied in male Wistar rat lung and liver microsomal preparations. All four antibodies decreased styrene metabolism in microsomes from rat liver, with anti-CYP2C11/6 having the strongest effect, but only anti-CYP2B1/2 affected the metabolism in lung microsomes. This indicates that the major CYP isoform responsible for metabolism of styrene is different in these two tissues (Nakajima *et al*., 1994b). Studies with human cytochromes P450 ectopically expressed in cultured hepatoma G2 cells indicate that CYP2B6 and CYP2F1 may also be involved in styrene metabolism (Nakajima *et al*., 1994a). The potential contributions of various cytochromes P450 to styrene metabolism have also been examined by use of chemical inhibitors of specific isozymes in hepatic and pulmonary microsomal preparations of male CD-1 mice. Diethyldithiocarbamate inhibited the formation of both enantiomers of styrene 7,8-oxide in lung and liver, supporting the importance of CYP2E1. 5-Phenyl-1-pentyne showed a

high degree of inhibition in pulmonary microsomes, but caused only a small decrease in hepatic microsomes, indicating the importance in mouse lung of CYP2F2 (Carlson, 1997a). α-Naphthoflavone, an inhibitor of CYP1A, had little effect on styrene metabolism. α-Methylbenzylaminobenzotriazole caused only a 16–19% inhibition of styrene 7,8-oxide formation at a concentration (1 μM) that caused substantial (87%) inhibition of benzyloxyresorufin metabolism, indicating that CYP2B plays a minor role in styrene metabolism (Carlson *et al.*, 1998).

Green *et al.* (2001a) examined the effects of 5-phenyl-1-pentyne (100 mg/kg bw) — an inhibitor of CYP2E1 and CYP2F2 — on the metabolism of styrene *in vitro* by pulmonary microsomes from male CD-1 mice isolated at various times up to 48 h after dosing. After six hours, styrene metabolism was reduced to 8% of control activity for the formation of the *R*-enantiomer of styrene 7,8-oxide and to 25% for the *S*-enantiomer. A single dose of 1 g/kg diethyldithiocarbamate inhibited the pulmonary microsomal metabolism of styrene to 16% of control activity for *R*- and 23% for *S*-styrene 7,8-oxide at 24 h after dosing. These studies further indicate the importance of the enzymes CYP2E1 and CYP2F2 in the pulmonary metabolism of styrene. Green *et al.* (2001b) carried out additional studies on the metabolism of styrene by nasal epithelium of CD-1 mice and Sprague-Dawley rats. The rates of formation of styrene 7,8-oxide were similar in microsomal preparations of the olfactory epithelium of mice and rats, with values of 6.59 and 7.46 nmol/min/mg protein, respectively, for the *R*-isomer and 2.24 and 2.51 nmol/min/mg protein for the *S*-isomer. Activity was approximately 50% lower in the respiratory epithelium than in the olfactory epithelium. No styrene metabolism was detected in nine samples of human nasal tissue (limit of detection: 0.04 nmol/min/mg protein). Both chlorzoxazone and 5-phenyl-1-pentyne inhibited styrene metabolism, again indicating the importance of the enzymes CYP2E1 and CYP2F2.

Recent studies have addressed the question of which pulmonary cell types are responsible for styrene metabolism and which cytochromes P450 are associated with the bioactivation of styrene. In enriched fractions of Clara cells and type II cells from male CD-1 mice and male Sprague-Dawley rats, styrene metabolism was determined with and without chemical inhibitors. Mouse Clara cells readily metabolized styrene to styrene 7,8-oxide, but type II pneumocytes had little or no activity. Styrene metabolism was several-fold higher in mouse Clara cells than in rat Clara cells. *R*-Styrene 7,8-oxide was preferentially formed in mouse lung and Clara cells, and *S*-styrene 7,8-oxide was preferentially formed in rat lung and Clara cells (Table 11) (Hynes *et al.*, 1999). Studies with both microsomes and isolated cells indicated that CYP2E1 and CYP2F2 are the main cytochromes P450 involved in pulmonary styrene metabolism. Green *et al.* (2001b) similarly found that hepatic microsomal fractions isolated from Sprague-Dawley rats preferentially formed *S*-styrene 7,8-oxide (ratio *R*:*S*, 0.72), whereas microsomal fractions prepared from CD-1 mice preferentially formed *R*-styrene 7,8-oxide (ratio *R*:*S*, 2.70). In olfactory and respiratory nasal cells, the rates of this bioactivation step were similar in both species and were higher in the respiratory epithelium than in the olfactory cells. Both chlorzoxazone (specific for CYP2E1) and 5-phenyl-1-pentyne (inhibitor of

Table 11. Metabolism of styrene to styrene 7,8-oxide enantiomers by mouse and rat lung microsomes and isolated Clara cell-enriched fraction

	R-enantiomer	*S*-enantiomer	*R*/*S*
Lung microsomes			
Mouse (n = 4)	1.50 ± 0.23[a]	0.63 ± 0.06	2.40 ± 0.36
Rat (n = 5)	0.49 ± 0.09	0.95 ± 0.18	0.52 ± 0.01
Clara cell fraction			
Mouse (n = 4)	83.3 ± 27.7	23.0 ± 8.2	3.98 ± 0.75
Rat (n = 3)	11.2 ± 3.6	11.0 ± 3.2	1.02 ± 0.09

Data from Hynes *et al.* (1999)
Values for *R*- and *S*-enantiomers are in nmol/mg protein/min for lung microsomes and in pmol/10^6 cells/min (not corrected for cell type) for Clara cell fractions. The percentages of Clara cells in these fractions were 56% for mouse and 37% for rat.
[a] Mean ± standard error

CYP2E1 and CYP2F2) inhibited nasal metabolism. Subsequent metabolism of styrene 7,8-oxide by epoxide hydrolase and GST was much higher in rat nasal tissue than in mouse nasal tissue.

(*d*) *Excretion*

Sumner *et al*. (1997) compared the metabolism of [7-^{14}C]styrene administered by inhalation (250 ppm [1060 mg/m^3], 6 h per day for 1–5 days) to B6C3F$_1$ mice, CD-1 mice and Fischer 344 rats. Rats and CD-1 mice excreted the radioactivity faster than did B6C3F$_1$ mice following a single exposure, but the rates were similar for the three groups after repeated exposures on 3 or 5 days. Boogaard *et al*. (2000a) studied the disposition of [*ring*-U-^{14}C]styrene in Sprague-Dawley rats and CD-1 mice exposed by recirculating nose-only exposure (160 ppm [682 mg/m^3] for 6 h). Urinary excretion was the primary route of elimination (amounting to 75% of the retained styrene for rats and 63% for mice), and there was little quantitative difference between rats and mice except that mice exhaled $^{14}CO_2$ which amounted to 6.4–8.0% of the retained styrene, while for rats this was only 2%. Whole-body autoradiography showed significantly higher non-specific binding of radioactivity in mouse lung and nasal passages compared with rat lung.

4.1.3 *Pharmacokinetic modelling*

Ramsey and Young (1978) and Ramsey *et al*. (1980) analysed the pharmacokinetic profile of inhaled styrene in male rats [strain unspecified] and humans. In human volunteers exposed to 80 ppm [341 mg/m^3] for 6 h, styrene was cleared from the blood

according to a two-compartment linear pharmacokinetic model that was similar to that for rats. The model predicted that there would be no accumulation of styrene at this exposure concentration and upon repeated administration. Ramsey and Andersen (1984) developed a physiologically based pharmacokinetic model, which indicated that there was saturation of styrene metabolism at inhaled doses above 200 ppm [852 mg/m^3] in mice, rats and humans. At lower levels of exposure, the ratio of styrene concentration in blood to that in inhaled air is controlled by perfusion-limited metabolism.

Another physiologically based pharmacokinetic model was based on data collected by Mandrala *et al.* (1993) to predict concentration–time curves for styrene and styrene 7,8-oxide in blood and tissues (Csanády *et al.*, 1994). At low concentrations, styrene is rapidly removed from blood and the rate of metabolism is limited by the blood flow through the liver.

Cohen *et al.* (2002) developed a pharmacokinetic model viewing the lung as two compartments, the alveolar tract and the capillary bed. This model included both pulmonary and hepatic metabolism and predicted tissue or organ concentrations of styrene and styrene 7,8-oxide (both the *S*- and *R*-enantiomers, separately), under specified conditions. It was based on the Csanády *et al.* (1994) model, which only accounted for styrene metabolism by the liver. The authors indicated that the model has a substantial degree of uncertainty, however, because of inconsistencies between studies in reporting styrene 7,8-oxide levels in blood, which complicated the validation of the model.

Filser *et al*. (2002) compared the modelled concentrations of styrene 7,8-oxide in the lungs of the mouse, rat and human over a range of styrene concentrations assuming 6- or 8-h exposures. The highest concentrations of styrene 7,8-oxide were predicted for the mouse followed by the rat, with humans having by far the lowest concentration. These differences reflected the species differences in the pulmonary metabolism of styrene to styrene 7,8-oxide and subsequent detoxification of the oxide.

The most comprehensive modelling for styrene metabolism and styrene 7,8-oxide dosimetry comparing rodents and humans was undertaken by Sarangapani *et al.* (2002). It expanded upon the Csanády *et al.* (1994) model by incorporating information on the metabolic production of styrene 7,8-oxide and its decrease in the respiratory tract and was used to predict the concentrations of styrene and styrene 7,8-oxide in blood, liver and respiratory tract. This model predicts a ten-fold lower styrene 7,8-oxide concentration in the terminal bronchioles of rats than in mice exposed to identical concentrations of styrene. The model suggests that styrene 7,8-oxide concentrations in human bronchioles would be 100-fold lower than for the mouse.

Tornero-Velez and Rappaport (2001) modified the physiologically based pharmacokinetic model of Csanády *et al*. (1994) to compare the predicted contribution of styrene 7,8-oxide resulting from metabolism with that from direct exposure in the workplace. They tested their model against air and blood concentrations of styrene and styrene 7,8-oxide in 252 workers in the reinforced plastics industry. Due to efficient detoxification of the oxide formed in the liver, direct exposure via inhalation appeared to be a more important source of styrene 7,8-oxide than the bioactivation of inhaled styrene. The

model indicated that absorbed styrene 7,8-oxide contributed 3640 times more of this compound to the blood than an equivalent amount of inhaled and metabolized styrene.

4.2 Toxic effects

4.2.1 *Humans*

Exposure to styrene has been reported to cause irritation of the eyes, throat and respiratory tract (Lorimer *et al.*, 1976, 1978). Subjective health complaints were not reported in the glass fibre-reinforced plastics industry with concentrations of styrene below 105 mg/m^3 (Geuskens *et al.*, 1992).

There are a number of reports of central and peripheral nervous system effects in exposed workers. Some studies have reported decreased nerve conduction velocities in workers exposed to styrene (Lilis *et al.*, 1978; Rosén *et al.*, 1978; Cherry & Gautrin, 1990; Murata *et al.*, 1991; Štetkárová *et al*., 1993), whereas others have shown no changes at styrene concentrations below 100 ppm [430 mg/m^3] (Edling & Ekberg 1985; Triebig *et al*., 1985). Electroencephalographic (Seppäläinen & Härkönen, 1976; Matikainen *et al*., 1993), dopaminergic (Mutti *et al.*, 1984a; Arfini *et al.*, 1987; Checkoway *et al.*, 1992, 1994; Bergamaschi *et al*., 1996, 1997), functional (Lindström *et al*., 1976; Cherry *et al.*, 1980) and psychiatric anomalies (Flodin *et al.*, 1989; Edling *et al*., 1993) were observed in styrene-exposed workers compared with controls. Most of these effects were observed at concentrations of styrene of about 100 ppm [430 mg/m^3], although memory and neurobehavioural disturbances were noted at styrene concentrations ranging from 10 to 30 ppm [43–130 mg/m^3] and above (Flodin *et al.*, 1989; Letz *et al.*, 1990; Edling *et al*., 1993; Jégaden *et al*., 1993; Tsai & Chen, 1996; Viaene *et al*., 2001).

Thresholds for hearing were unchanged in workers exposed to styrene concentrations less than 150 mg/m^3. However, a comparison within the group of styrene-exposed workers (least exposed versus most exposed) showed a significant difference in hearing thresholds at high frequencies (Muijser *et al.*, 1988). In a group of 18 workers exposed for 6–15 years to styrene at levels below 110 mg/m^3, seven workers showed disturbances in the central auditory pathways (Möller *et al.*, 1990). Morioka *et al*. (1999) found a reduction in the upper limit of hearing in workers occupationally exposed to organic solvents. This effect was dose-dependent and correlated with the concentration of styrene in the breathing zone of the workers and with the amount of mandelic acid in urine. In a group of 299 workers in the glass fibre-reinforced plastics industry, there was no evidence for an effect of exposure to styrene on hearing acuity when both noise and lifetime exposures to styrene were accounted for (Sass-Kortsak *et al*., 1995).

Colour vision was found to be impaired in styrene-exposed workers in a number of studies (Gobba *et al*., 1991; Fallas *et al*., 1992; Chia *et al*., 1994; Campagna *et al*., 1995; Eguchi *et al*., 1995; Campagna *et al*., 1996; Gobba, 2000). Solvent-induced loss of colour vision is indicative of changes in neural functioning along optic pathways. A

positive relationship was observed between styrene exposure and early colour vision dysfunction in several of these studies, with effects detected at exposure concentrations as low as 20 ppm [85 mg/m^3]. In one study, a dose–response relationship between the concentration of mandelic acid in urine and colour vision loss was noted (Kishi *et al*., 2001). Triebig *et al*. (2001) showed that styrene-induced colour vision dysfunction is reversible when the person is in a styrene-free environment for four weeks.

In a study of mortality among styrene-exposed workers, an increased number of deaths attributed to 'symptoms, senility and ill-defined conditions' was ascribed to a high local registration of these conditions in comparison with national statistics (Bond *et al*., 1992). Welp *et al*. (1996a) followed a cohort of workers in the reinforced-plastics industry and found that mortality from diseases of the central nervous system, especially epilepsy, increased with exposure to styrene. Exposure was evaluated as duration (< 1–>10 years), average concentration (60–120 ppm [256–511 mg/m^3]) and cumulative exposure (< 50–> 500 ppm–years [< 213–> 2130 mg/m^3–years]). The effects of styrene on the respiratory tract of workers exposed to concentrations above 100 mg/m^3 include chronic bronchitis (Härkönen, 1977). Haematological changes (in blood coagulation and fibrinolysis) have been observed (Chmielewski & Renke, 1976). Cases of styrene-induced asthma (Moscato *et al*., 1987; Hayes *et al*., 1991) and one case of contact dermatitis (Sjöborg *et al*., 1984) have also been reported. Welp *et al*. (1996b) reported that mortality from pneumonia among more than 40 000 men and women in 660 European factories manufacturing reinforced plastics appeared to be associated with exposure to styrene, but mortality from bronchitis, emphysema and asthma was not. In a more recent study, no relationship was observed between exposure to styrene and mortality from non-malignant respiratory diseases among 15 826 workers exposed to styrene (average styrene concentrations were below 10 ppm [43 mg/m^3], with 14% of the workers exposed to ≥ 60 ppm [256 mg/m^3]) in the reinforced-plastics and composites industry in the USA (Wong & Trent, 1999).

Several studies have reported signs of liver damage, as measured by liver enzyme activities in serum, but it was concluded in a review that no clear trend towards altered liver function had been demonstrated (WHO, 1983). Elevated serum bile acid concentrations were observed in one study (Edling & Tagesson, 1984) but not in another (Härkönen *et al*., 1984).

More recently, two independent cross-sectional studies in 47 workers in the glass fibre-reinforced plastics industry and in 21 boat and tank fabricators, both with separate control groups, were carried out by Brodkin *et al*. (2001). Exposure to styrene was assessed by measurements in blood and air samples. Workers were grouped as controls, low-exposure (≤ 25.0 ppm [106 mg/m^3] styrene), or high-exposure (> 25.0 ppm styrene). Direct bilirubin levels, a marker of altered hepatic clearance, increased with styrene exposure. Hepatic transaminase activities (alanine and aspartate transaminases) increased with styrene exposure when data from both studies were pooled.

Altered kidney function in styrene-exposed workers was measured by increased urinary excretion of albumin (Askergren *et al*., 1981). Vyskocil *et al*. (1989) studied

female workers in the glass fibre-reinforced plastics industry who were exposed to styrene at 225 mg/m^3 for a mean duration of 11 years. No difference was found in the excretion of a number of urinary markers (including albumin) of renal injury compared with control female workers. Another study showed no difference in urinary markers for renal toxicity in workers exposed to styrene at ≤ 215 mg/m^3 for 1–13 years (mean, six years) compared with an unexposed control group (Viau *et al*., 1987). In ten workers exposed to styrene (8-h time-weighted average exposure estimated to be 21–405 mg/m^3) and 15 non-exposed workers, only a weak correlation was noted between styrene exposure concentration and alterations in urinary markers for renal toxicity (Verplanke & Herber, 1998).

Early studies on the effects of styrene on the haematopoietic and immune system did not consistently reveal changes (WHO, 1983). Thiess and Friedheim (1978) found no difference in haemoglobin, erythrocyte and leukocyte concentrations in 84 workers in styrene production, polymerization and processing plants exposed to styrene (50–500 ppm [213–2130 mg/m^3] for 1–36 years) compared with a reference group. In a study of 163 workers in a styrene–butadiene synthetic rubber manufacturing plant, there was no pronounced evidence of haematological abnormality, as determined from measurements taken in peripheral blood, from exposure to styrene (< 15 ppm [64 mg/m^3]) (Checkoway & Williams, 1982). In a group of workers exposed to 56 mg/m^3 styrene in the reinforced-plastics industry, a 30% increase in the number of peripheral blood monocytes was noted (Hagmar *et al.*, 1989). In 221 styrene-exposed (1–100 ppm [4.3–426 mg/m^3] for 1–20 years) workers in the reinforced-plastics industry, an exposure-related decrease was detected in the mean corpuscular haemoglobin and neutrophil concentrations compared with the values in 104 controls (Stengel *et al*., 1990). Mean corpuscular volume and monocytes increased with exposure to styrene. No changes were found in haemoglobin, red blood cell, white blood cell, lymphocyte or platelet concentrations in the styrene-exposed workers compared with the unexposed group. In another group of 32 workers exposed to styrene, changes in lymphocyte subpopulations were observed mainly at exposure levels above 50 ppm (Mutti *et al.*, 1992). Bergamaschi *et al*. (1995) reported a reduction in total T lymphocytes (CD3$^+$) and T helper lymphocytes (CD4$^+$) and an increase in natural killer cells in styrene-exposed workers (10–50 ppm [43–213 mg/m^3], 8-h time-weighted average). An association was observed between styrene exposure of hand-lamination workers (mean blood styrene levels of 946 μg/L) and alterations of cell-mediated immune response of T lymphocytes, with an imbalance of leukocyte subsets (increased number of monocytes and decreased number of lymphocytes) in the peripheral blood (Tulinska *et al*., 2000).

4.2.2 *Experimental systems*

Single exposures of rats and guinea-pigs [strain not stated] to 1300 ppm [5630 mg/m^3] styrene resulted in central nervous system effects, including weakness and unsteadiness. After exposure to 2500 ppm [10.8 g/m^3] styrene for 10 h, both rats and

guinea-pigs lost consciousness; exposure to 5000–10 000 ppm [21.3–42.6 g/m^3] resulted in unconsciousness and death. The principal pathological findings in these animals were severe pulmonary irritation, congestion, oedema, haemorrhage and leukocytic infiltration (Spencer *et al*., 1942).

Early studies on the neurotoxicity of styrene gave equivocal results (WHO, 1983), but a decrease in the activity of monoamine oxidase in brain was seen in male rats after repeated oral doses of styrene (1.2 g/kg bw per day for seven days) (Zaprianov & Bainova, 1979). In subsequent studies, exposure of male rabbits to styrene vapour (0, 750 or 1500 ppm [0, 3200 or 6400 mg/m^3] for 12 h per day during three or seven days) caused a dose-dependent decrease in striatal and tuberoinfundibular dopamine content and an increase in homovanillic acid content, consistent with disturbance of the dopaminergic functions of the brain (Mutti *et al.*, 1984b). The levels of norepinephrine were unchanged. The styrene metabolites phenylglyoxylic acid and mandelic acid were shown to deplete dopamine through a direct chemical reaction, thereby rendering it inactive as a neurotransmitter (Mutti *et al.*, 1988). Chakrabarti (2000) administered styrene at 0.25 and 0.5 mg/kg bw by gavage to male Sprague-Dawley rats on seven days per week for 13 weeks; there was a decrease in dopamine and its metabolites in the corpus striatum, hypothalamus and the lateral olfactory tract regions in the high-dose group. A loss of motor function that accompanied these changes lasted for approximately a month after the last dose. Styrene (300 ppm [1280 mg/m^3], 6 h per day, five days per week for 12 weeks) also caused cell loss and dopamine depletion in retinas isolated from female Sprague-Dawley rats (Vettori *et al*., 2000).

An increase in the concentration of glial fibrillary acidic protein was measured in the sensory motor cortex and in the hippocampus of Sprague-Dawley rats that had been continuously exposed to 320 ppm [1390 mg/m^3] styrene for three months and then kept free from exposure for another four months. This effect was taken to be an indication of solvent-induced brain damage (Rosengren & Haglid, 1989). Mild neurobehavioural disturbances were seen in rats exposed to 1400 ppm [6070 mg/m^3] styrene for 16 h per day, five days per week for 18 weeks followed by a six-week exposure-free period (Kulig, 1989) and in mice exposed to 425 ppm [1840 mg/m^3] styrene for 4 h per day, five days per week for two weeks (Teramoto *et al.*, 1988).

In dissociated primary cultures of murine spinal cord–dorsal root ganglia–skeletal muscle, styrene and styrene 7,8-oxide were cytotoxic to neuronal and non-neuronal cells at concentrations in excess of 2 and 0.2 mM, respectively (Kohn *et al.*, 1995).

Styrene is ototoxic in rats (Pryor *et al*., 1987; Yano *et al*., 1992; Crofton *et al*., 1994). Exposure of male Long-Evans rats to 850 ppm and 1000 ppm [3620 and 4260 mg/m^3] styrene for 6 h per day, five days per week for four weeks, caused permanent hearing loss at mid-frequency ranges (16 kHz). At higher exposure concentrations, the increase in the auditory threshold became frequency-independent and was observed in the mid-low, mid- and high-frequency ranges (Loquet *et al*., 1999).

Styrene suppressed the activity of mouse splenic T lymphocyte killer cells *in vitro* (Grayson & Gill, 1986). When Wistar rats were exposed to styrene (210 mg/m^3)

continuously for seven days, δ-aminolaevulinate dehydratase activity was inhibited in erythrocytes and bone marrow (Fujita *et al.*, 1987). Male Swiss mice dosed orally with 20–50 mg/kg bw styrene daily for five days showed impairment of humoral and cell-mediated immunity (Dogra *et al.*, 1989); a similar dose regimen given for four weeks decreased the resistance of mice to viral, malarial and hookworm infections (Dogra *et al.*, 1992). Male Sprague-Dawley rats given styrene by intraperitoneal injection (400 mg/kg bw per day for three days) or exposed by inhalation (300 ppm [1280 mg/m^3], 6 h per day, five days per week for two weeks) showed an increased number of erythropoietic cells. The granulocytopoietic series was not affected by injected styrene but was depressed following longer-term inhalation exposure (Nano *et al.*, 2000).

Ohashi *et al.* (1985) investigated the respiratory toxicity of styrene in rats. Epithelial changes were observed in the nose and trachea of rats exposed to 800 ppm [3410 mg/m^3] styrene for 4 h per day during eight weeks followed by a three-week period without exposure. The changes included vacuolation of epithelial cells, nuclear pyknosis and exfoliation of epithelial cells. Mild changes in the nasal mucosa occurred at exposure levels of 30 ppm [130 mg/m^3]. Morphological damage was more severe in the upper than in the lower respiratory tract.

In Swiss-Albino mice, intraperitoneal injections of a high dose of styrene (800 mg/kg bw) or its metabolite styrene 7,8-oxide (300 mg/kg bw) induced an increase in γ-glutamyl transpeptidase and lactate dehydrogenase measured in bronchoalveolar lavage fluid. These enzymes are biomarkers of pulmonary toxicity (Gadberry *et al.*, 1996). Treatment of male Sprague-Dawley rats with styrene either by intraperitoneal injection (40 or 400 mg/kg bw, daily for three days) or by inhalation exposure (300 ppm [1280 mg/m^3], 6 h per day, five days per week for two weeks) showed the same pattern of cytoplasmic changes involving bronchiolar and alveolar type II cells as observed in mice (Coccini *et al.*, 1997). Damage in the lung was accompanied by glutathione depletion. Damage was more severe following intraperitoneal injection compared with inhalation exposure. Male CD-1 mice were exposed to styrene at 0, 40 and 160 ppm [170 and 682 mg/m^3], and male Sprague-Dawley rats to 0 and 500 ppm [2130 mg/m^3] for 6 h per day for up to 10 days (Green *et al.*, 2001a). In mice, styrene exposure caused pulmonary toxicity characterized by focal loss of cytoplasm and focal crowding of non-ciliated Clara cells, particularly in the terminal bronchiolar region. The toxicity was accompanied by an increase in cell replication rates in terminal and large bronchioles of mice exposed for three days or longer. Morphological and cell-proliferative effects were not observed in the alveolar region of the mouse lung or in the lungs of rats exposed to 500 ppm styrene. 5-Phenyl-1-pentyne, an inhibitor of cytochrome P450-mediated metabolism, was given to a group of mice before styrene exposure. Cell replication rates in the lungs of these mice were similar to those in control mice, suggesting that the pulmonary effects are due to a styrene metabolite.

Khanna *et al.* (1994) examined pancreatic damage following oral administration of styrene to mice (25 or 50 mg/kg bw) and rats or guinea-pigs (160 or 320 mg/kg bw) on five days per week for four weeks. In all three species, styrene caused congestion of

pancreatic lobules, inflammatory reactions around pancreatic islets (in mice only), and altered serum insulin levels but no change in blood glucose levels.

Early studies reported morphological changes in the kidney of Sprague-Dawley rats after intraperitoneal injections of styrene (2.9 and 5.8 mmol/kg bw on five days per week for six weeks) (Chakrabarti *et al.*, 1987) and in the respiratory mucosa of SD rats exposed to styrene by inhalation (150 or 1000 ppm [639–4260 mg/m^3], 4 h per day, five days per week, three weeks) (Ohashi *et al.*, 1986). Effects in kidney and lung are associated with depletion of glutathione (Chakrabarti & Tuchweber, 1987; Elovaara *et al.*, 1990) and possibly with direct toxicity of glutathione conjugates to the kidney, as was shown in experiments with a synthetic glutathione–styrene conjugate (Chakrabarti & Malick, 1991). Viau *et al.* (1987) reported that Sprague-Dawley rats given intraperitoneal injections of styrene (1 g/kg bw, which is one-fifth the oral LD_{50}) daily for 10 days showed only mild tubular damage. In female Sprague-Dawley rats exposed to styrene (300 ppm [1280 mg/m^3], 6 h per day, five days per week, for 12 weeks), a small increase was observed in the urinary excretion of plasma proteins, with minor changes in kidney histopathology consisting of interstitial fibrosis, cystic dilatations and hyaline tubules (Mutti *et al*., 1999).

Early studies reported that styrene caused hepatotoxicity in rats concomitantly with a depletion of glutathione, which may be either a direct effect of styrene or mediated by lipid peroxidation (Srivastava *et al.*, 1983; Katoh *et al.*, 1989). Male albino rats [strain not stated] were dosed orally with 200 or 400 mg/kg bw styrene on six days per week for 100 days (Srivastava *et al*., 1982). Liver changes were observed only after the high dose and were characterized by tiny areas of focal necrosis composed of a small number of degenerated hepatocytes and inflammatory cells.

Exposure of B6C3F$_1$ mice to 0, 125, 250 or 500 ppm [0, 530, 1060 or 2130 mg/m^3] styrene for 6 h per day for 14 days caused severe centrilobular hepatic necrosis and death after one 6-h exposure to 500 ppm or two 6-h exposures to 250 ppm styrene (Morgan *et al*., 1993a). Mortality was higher in male than in female mice and, after 14 days of exposure, the incidence and severity of liver lesions were also greater in male mice. In a subsequent study, the greater sensitivity of male mice to styrene exposure compared with females could not be explained by differences in glutathione depletion or styrene or styrene 7,8-oxide concentrations in blood (Morgan *et al*., 1993b). Further investigation of the role of metabolism in the toxicity of styrene in mice revealed that styrene and styrene 7,8-oxide concentrations in blood did not correlate well with strain differences in sensitivity (Morgan *et al*., 1993c). Mortality and hepatotoxicity induced by exposure to styrene were greater in B6C3F$_1$ and C57BL/6 mice than in Swiss mice, while DBA/2 mice were the least sensitive. Styrene and styrene 7,8-oxide concentrations in blood as well as glutathione depletion were similar in B6C3F$_1$ and DBA/2 mice but greater than those in Swiss mice. Although strain and gender differences in sensitivity to styrene toxicity have been suggested to be due to differences in metabolism, styrene 7,8-oxide concentrations in blood did not correlate with toxicity in these studies. Morgan *et al.* (1995) further investigated strain and gender differences in styrene toxicity at inhalation

exposure concentrations below the saturation of metabolism (150 and 200 ppm [639 and 852 mg/m^3] styrene, 6 h per day, five days per week, for two weeks). Female B6C3F$_1$ mice were more susceptible than male B6C3F$_1$ and male or female Swiss mice to the hepatotoxic effects of styrene. This was suggested to be due to the fact that styrene caused greater depletion of glutathione in female B6C3F$_1$ mice, which also had a lower capacity to regenerate glutathione. Morgan *et al*. (1997) showed that hepatotoxicity in male B6C3F$_1$ mice that had been treated with phenobarbital before a 6-h exposure to 500 ppm [2130 mg/m^3] styrene was more severe than in control mice treated with styrene alone. In male ddY mice pretreated with buthionine sulfoximine, a glutathione depletor, oral administration of styrene (0.96–5.76 mmol/kg) or styrene 7,8-oxide (3.84 mmol/kg) caused hepatotoxicity characterized by centrilobular necrosis and an increase in serum alanine transaminase (Mizutani *et al*., 1994).

Cruzan *et al*. (1997) carried out several subchronic inhalation studies with styrene in Sprague-Dawley rats and CD-1 and B6C3F$_1$ mice. In rats exposed to 200–1500 ppm [852–6390 mg/m^3] styrene (6 h per day, five days per week) for 13 weeks, styrene caused treatment-related lesions in the olfactory epithelium of the nasal passages at doses ≥ 500 ppm [2130 mg/m^3], which were characterized by focal disorganization and hyperplasia of basal cells, single-cell necrosis and apparent cell loss. No increase in cell proliferation was observed in liver or lung following exposure to styrene for 2, 5 or 13 weeks. When CD-1 and B6C3F$_1$ mice were exposed to styrene at concentrations ranging from 15 to 500 ppm [64–2130 mg/m^3] (6 h per day, five days per week) for two weeks, exposure to 250 or 500 ppm [1060 or 2130 mg/m^3] caused mortality in both strains, together with hepatotoxic effects, which could be characterized microscopically as centrilobular hepatocyte necrosis. Mortality and liver changes were more severe in female mice at 250 ppm than at 500 ppm styrene. In a 13-week inhalation study, CD-1 mice were exposed to styrene (6 h per day, five days per week), with concentrations ranging from 50 to 200 ppm [213–852 mg/m^3]. Liver toxicity was evident, with females generally more severely affected than males. Atrophy of the olfactory epithelium and olfactory nerve fibres occurred with or without focal respiratory metaplasia. In the lungs of a majority of exposed mice in all dose groups, decreased eosinophilia of the bronchial epithelium occurred. Cell proliferation studies revealed a highly variable labelling index in different cell types and between animals. There was a statistically significant increase in proliferation of the Clara cells of mice after two weeks of exposure to 150 and 200 ppm [639 and 852 mg/m^3] styrene. These studies showed that mice are more sensitive to the toxic effects of styrene than rats. When Sprague-Dawley rats were exposed to styrene (50–1000 ppm [213–4260 mg/m^3], 6 h per day, five days per week for 104 weeks), no changes in haematology, clinical chemistry, urinalysis or organ weights were observed (Cruzan *et al*., 1998). However, the incidence of lesions of the olfactory epithelium of the nasal mucosa increased with increasing exposure concentration.

Male non-Swiss Albino mice were given single intraperitoneal injections of styrene (250, 500 or 1000 mg/kg bw), racemic styrene 7,8-oxide (100, 200, 300 or 400 mg/kg) or *R*- or *S*-styrene 7,8-oxide (300 mg/kg bw) and killed 24 h later (Gadberry *et al*., 1996).

Styrene caused a dose-dependent increase in serum sorbitol dehydrogenase activity, an indicator of hepatotoxicity. Pretreatment of the animals with pyridine and β-naphthoflavone, inducers of CYP enzymes, increased the toxicity of styrene, but pretreatment with phenobarbital had no such effect. Trichloropropene oxide, an inhibitor of epoxide hydrolase, increased the hepatotoxicity of styrene 7,8-oxide, while buthionine sulfoximine, a glutathione depletor, did not. When given at the same dose (300 mg/kg bw), the racemic mixture of styrene 7,8-oxides and *R*-styrene 7,8-oxide caused a greater hepatotoxic effect than did *S*-styrene 7,8-oxide. However, differences in pulmonary toxicity between the enantiomers were not statistically significant. In a subsequent study to assess the susceptibility of mouse strains to the effects of styrene and styrene 7,8-oxide, male mice (A/J, C57BL/6 or CD-1) were given an intraperitoneal injection of 800 mg/kg bw styrene and killed 24 h later (Carlson, 1997b). Biomarkers of hepatotoxicity and pneumotoxicity were evaluated as in the study mentioned above (Gadberry *et al.*, 1996). CD-1 mice were not as sensitive as the other strains to the hepatotoxic or pneumotoxic effects of styrene, but were equally sensitive to the toxic effects of styrene 7,8-oxide given by intraperitoneal injection at 300 mg/kg bw.

Hepatotoxicity and cell proliferation induced in male B6C3F_1 mice by a single 6-h exposure to 500 ppm [2130 mg/m^3] styrene was compared with that caused by repeated exposures to 500 ppm styrene for 6 h per day for up to 14 days (Mahler *et al.*, 1999). Single or repeated inhalation exposure of mice to styrene caused severe necrosis of centrilobular hepatocytes followed by regeneration of necrotic zones. Re-exposure to styrene of mice that had received only a single dose of styrene resulted in hepatocellular necrosis, indicating that regenerated centrilobular cells were again susceptible to the cytolethal effect of styrene following a 14-day recovery. In contrast, continuous styrene exposure induced hepatocellular resistance to the cytolethal effect of styrene. New hepatocytes were produced with reduced metabolic activity. Centrilobular hepatocytes were resistant to styrene while they were actively synthesizing DNA and continued styrene exposure was required for sustained DNA synthesis.

4.3 Reproductive and developmental effects

Reproductive and developmental effects of styrene have been reviewed by IARC (1994a) and very extensively by Brown *et al.* (2000). The present evaluation is mainly based on these reviews and recent studies not included therein.

4.3.1 *Humans*

Early human studies suggested an association between occupational exposure to styrene and congenital central nervous system malformation (Holmberg, 1977, 1979), but subsequent, more extensive studies by the same and other investigators did not confirm the association (Brown *et al.*, 2000).

The frequency of spontaneous abortions among women with definite or assumed occupational exposure to styrene has been investigated. An early study suggested an association between exposure to styrene and spontaneous abortions (Hemminki *et al.*, 1980), but several later investigations, by the same group and others, of greater population size failed to confirm the association (see, e.g., Lindbohm *et al.*, 1985). It is possible to conclude that styrene is not associated with a major increase in the occurrence of spontaneous abortions, but because of the relatively small study population sizes in all of the investigations, the possibility of a small increase in risk cannot be excluded (Brown *et al.*, 2000).

There are only few studies on potential effects of exposure to styrene on birth weight. One study suggested that exposure to multiple solvents, including exposure to styrene levels above 80 ppm [340 mg/m^3], may be associated with around 4% reduced birth weight; however, the difference was not statistically significant (Lemasters *et al.*, 1989; Brown *et al*., 2000).

Data regarding the effects of styrene on the menstrual cycle are conflicting. Abnormalities in pituitary secretion in women exposed to styrene have been suggested and may be connected with putative effects on the menstrual cycle (Brown *et al*., 2000). In a recent study, exposure to aromatic solvents including styrene was associated with a trend towards increased frequency of oligomenorrhoea (average menstrual cycle length, > 35 days). Individually, exposure to styrene showed the greatest increase in odds ratio among the solvents (1.7; 95% CI, 1.1–2.6) and this increase was statistically significant (Cho *et al.*, 2001).

One study (Jelnes, 1988) suggested an increase in sperm abnormalities in workers exposed to high levels of styrene in the reinforced-plastics industry, but the data are weak and no firm conclusions can be drawn (Brown *et al*., 2000). In a later study, semen samples were collected from 23 workers during the first week of employment in the reinforced-plastics industry and after six months of exposure to styrene and from 21 non-exposed farmers. A significant decline in sperm density was seen during exposure to styrene, whereas no decline was seen in the non-exposed men. No indication of an exposure–response relationship was seen when individual changes in semen quality within the group of reinforced-plastics workers were related to post-shift urinary mandelic acid concentrations, controlled for change in potential time-dependent confounders. However, the exposure gradient in the group was modest. The earlier finding of an increase in sperm abnormalities was not corroborated by this study (Kolstad *et al*., 1999).

Among 1560 male workers in the reinforced-plastics industry in Denmark, Italy and The Netherlands, 220 styrene-exposed workers and 382 unexposed referents who had fathered a child were identified. The relationship between occupational exposure to styrene of these men and time-to-pregnancy of their partners was investigated. No consistent pattern of reduced male fecundity was found when the time to pregnancy was related to work tasks that involved higher levels of exposure to styrene or for which semiquantitative or quantitative measures of exposure to styrene were available. The workers with high exposure levels showed a fecundity ratio of 1.1 (95% CI, 0.69–1.7).

On the basis of these results, the authors concluded that it is unlikely that exposure to styrene has a strong effect on male fecundity (Kolstad *et al*., 2000).

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity studies*

Styrene has been shown to cross the placenta in rats and mice. In rats, fetal styrene concentrations of around 50% of the levels in maternal blood have been found (Withey & Karpinski, 1985; IARC, 1994a).

The potential developmental toxicity of styrene has been tested in several experimental animals including rats, mice, rabbits and hamsters. Throughout the studies, there was no evidence for an increased incidence of malformations. There have been reports of increases in embryonic, fetal and neonatal death, and possibly skeletal and kidney abnormalities at inhalation exposure levels of around 250 ppm [1060 mg/m^3] and higher during pregnancy. These dose levels also caused some effects on the dams such as decreased maternal weight gain (IARC, 1994a; Brown *et al.*, 2000).

Decreased pup weight, postnatal developmental delays as well as neurobehavioural and neurochemical abnormalities have been reported in rats exposed by inhalation to styrene prenatally and/or postnatally (Brown *et al.*, 2000). In a recent study, rats (JCL: Wistar) were exposed to 0, 50 or 300 ppm [213 or 1280 mg/m^3] styrene for 6 h per day during gestation days 6–20 and the offspring were investigated postnatally for neurochemical changes, growth and physical landmarks of development. In order to adjust for nutrient conditions, pair-fed and ad-libitum controls were included. No significant difference in maternal weight gain was observed compared with either control group, but food consumption was decreased at the 300-ppm exposure level. An increased neonatal death rate was found at 300 ppm styrene compared with the pair-fed control group. Litter size and birth weight were not affected, while postnatal development (incisor eruption, eye opening) was delayed at 300 ppm compared with both control groups. In addition, neurochemical alterations indicated by decreased 5-hydroxytryptamine turnover were detected at postnatal day 21 in offspring exposed *in utero* to 300 ppm compared with both control groups (Katakura *et al.*, 2001). The findings in this study can be attributed to exposure to styrene rather than to insufficient nutrition due to the use of a pair-fed control group and thus the results support previous findings indicating that postnatal development and the developing brain can be affected by exposure to styrene.

In mice exposed to 100 ppm [426 mg/m^3] styrene continuously for 24 h during days 0–15 of gestation, lower fetal and placental weight as well as reduced maternal body weight gain were found (Ninomiya *et al.*, 2000).

(*b*) *Reproductive toxicity studies*

No effects on fertility were found in male and female rats exposed continuously for three generations to 125 or 250 ppm [532 or 1060 mg/m^3] styrene in the drinking-water (Beliles *et al.*, 1985). Water consumption was significantly reduced in both treatment

groups. Only minor general toxic effects (slightly reduced body weight in females) were observed. [The Working Group noted that the negative results obtained in this study do not provide adequate assurance of an absence of potential to impair fertility after exposure to styrene at higher dose levels and by other routes.]

No effects on sperm head morphology or sperm development were found in male mice exposed to 300 ppm [1280 mg/m^3] styrene (6 h per day, five days per week, for five weeks) (Salomaa *et al.*, 1985). General studies of the chronic and subchronic toxicity of styrene in several species have not shown testicular pathology at inhalation concentrations of up to 160 ppm [682 mg/m^3] in mice and 1000 ppm [4260 mg/m^3] or higher in other species (rat, rabbit, guinea-pig, rhesus monkey) (Brown *et al.*, 2000). One study in rats found evidence of testicular toxicity (decreased sperm count and some changes in testicular pathology) at oral styrene doses of 400 mg/kg bw per day for 60 days (Srivastava *et al.*, 1989). Srivastava *et al.* (1992) showed similar decreases in sperm count and testicular enzymes in postnatally maturing rats after treatment with styrene (200 mg/kg bw but not 100 mg/kg bw per day) by gavage for the first 60 days of life.

In peripubertal male C57BL/6 mice, free testosterone concentrations in plasma were strongly reduced after four weeks of exposure to styrene in the drinking-water (50 mg/L; this resulted in a daily intake of styrene of approximately 12 mg/kg bw). There were no effects on body weight, testis weight or plasma corticosterone and luteinizing hormone levels (Takao *et al.*, 2000).

There are only limited data on potential effects of styrene on estrous cyclicity. One study reported increased estrus cycle length and decreased body weight in rats exposed to 11.6 ppm [49 mg/m^3] styrene (Iziumova, 1972). However, the reporting of this study is confusing, the duration of estrus was poorly defined, and it is not clear if a comparable (sham) control group was used. In addition, it seems unlikely based on other studies of styrene that such a low exposure level would cause effects on body weight (see comments in Brown *et al.*, 2000).

The potential developmental toxicity of styrene and styrene 7,8-oxide was investigated in two in-vitro culture systems: micromass cell cultures of rat embryo midbrain or limb-bud cells and whole-embryo culture. Effects of styrene 7,8-oxide on markers of differentiation of limb-bud cells were evident at concentrations that had minimal effect on cell viability. Styrene alone or in the presence of an exogenous mono-oxygenase system had minimal effects even when tested at much higher concentrations than styrene 7,8-oxide. In whole-embryo culture, styrene 7,8-oxide caused growth retardation and malformations at somewhat lower levels than those producing embryo mortality (Gregotti *et al.*, 1994). In another study using postimplantation rat embryos in vitro, styrene 7,8-oxide was embryotoxic at concentrations more than 20 times lower than styrene (Brown-Woodman *et al.*, 1994).

4.4 Genetic and related effects

4.4.1 *Styrene*

(*a*) *Humans*

The genetic toxicology of styrene in humans was previously evaluated (IARC, 1994a). In styrene-exposed workers, levels of O^6-guanine adducts in lymphocyte DNA are up to five times higher than those in non-exposed controls. Variable results have been reported with regard to the association between exposure to styrene and chromosomal damage. Increased frequencies of chromosomal aberrations *in vivo* were not reported in most studies and only weak evidence was reported for induction of sister chromatid exchange. In-vitro studies consistently reported increased frequencies of chromosomal aberrations and sister chromatid exchange in human lymphocytes. Styrene did not induce mutations in bacteria, except in some studies that used exogenous metabolic activation. Most of the data published since the previous evaluation focus on the occurrence of DNA adducts, other DNA damage and cytogenetic effects associated with occupational exposures to styrene, and are summarized below.

(i) *DNA adducts*

Vodicka *et al.* (1993) studied a group of lamination workers exposed to styrene at air concentrations of 300–700 mg/m^3 (mean, 370 mg/m^3) and reported a statistically significant increase in the formation of O^6-deoxyguanosine adducts (O^6-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine, O^6-dG-styrene) in their lymphocytes, as determined by ^{32}P-postlabelling.

The persistence of these O^6-dG-styrene DNA adducts was investigated with a modified ^{32}P-postlabelling method (Hemminki & Vodicka, 1995), by comparing adduct levels in workers before vacation, after two weeks of vacation and after an additional one month of work. Air sampling showed styrene concentrations of 40–225 mg/m^3 (mean 122 mg/m^3). There was no significant difference in adduct levels in granulocytes between the control and exposed groups in any individual samplings. In lymphocytes of the laminators, the adduct levels were significantly higher (5.4 adducts/10^8 nucleotides) than those in the controls (1.0 adduct/10^8 nucleotides). A two-week interruption of exposure did not influence the total adduct level (4.9 versus 5.1 adducts/10^8 nucleotides in the first and second samplings, respectively), which indicates a very slow removal from the DNA of the specific O^6-dG-styrene adducts (Vodicka *et al.*, 1994).

Personal dosimeters were used to monitor exposure to styrene of nine workers at a hand-lamination plant in Bohemia, and the concentrations of styrene in blood and mandelic acid in urine were measured. Blood samples taken at four occasions during a seven-month period were analysed for styrene-specific O^6-dG adducts in lymphocytes and granulocytes. DNA strand breaks and hypoxanthine guanine phosphoribosyltransferase (*HPRT*) mutant frequency in T lymphocytes were also measured (see next section). Seven administrative employees in the same factory (factory controls) and eight persons in a research laboratory (laboratory controls) were used as referents. The mean

concentrations of styrene in air were 122 and 91 mg/m^3, for the first and fourth samplings, respectively. DNA adduct levels were determined by the ^{32}P-postlabelling method in lymphocytes of laminators, and appeared to be remarkably constant (5–6 adducts/10^8 nucleotides) and significantly higher ($p < 0.0001$) than those in factory controls (0.3–1 adduct/10^8 nucleotides) at all four sampling times (Vodicka *et al.*, 1995).

Results of a three-year follow-up study continued to show significant increases in O^6-dG-styrene adducts in lymphocytes of lamination workers exposed to styrene (5.9 ± 4.9 adducts/10^8 nucleotides versus 0.7 ± 0.8 adducts/10^8 nucleotides in controls) (Vodicka *et al.*, 1999). In this series of studies, the numbers of exposed workers and corresponding control subjects ranged from nine to 17 individuals. The results indicate that DNA adduct levels in lamination workers correlate with styrene exposure concentration. Evidence from long-term studies, however, indicates that adducts do not continue to accumulate with exposure time, suggesting that a balance is reached between adduct formation and removal during long-term exposures. Smoking habits did not significantly affect adduct formation in the exposed workers compared to controls.

In workers with reinforced plastics in the boat-manufacturing industry, styrene exposure (1–235 mg/m^3; mean 65.6 mg/m^3) increased the level of an unidentified DNA adduct (14.2 ± 2.3 adducts/10^8 nucleotides), as well as N^2-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine (15.8 ± 3.2 adducts/10^8 nucleotides) (Horvath *et al*., 1994; Rappaport *et al.*, 1996). Styrene exposure concentration correlated with N^2-guanine adduct levels. The increased formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) adducts in lymphocytes of styrene-exposed workers (2.23 ± 0.54 8-OHdG/10^5 dG versus 1.52 ± 0.45 8-OHdG/10^5 dG in controls) provides evidence that styrene exposure also causes oxidative DNA damage (Marczynski *et al.*, 1997a). It was suggested that such damage could alter the balance of oxidants and antioxidants in the cell. This has led to a new hypothesis for genotoxicity of styrene based on oxidative stress (Marczynski *et al*., 2000).

(ii) *DNA single-strand breaks*

In 17 workers with low occupational exposure to styrene (time-weighted average exposure, 29.4 mg/m^3), an exposure-dependent increase was seen in single-strand breaks (IARC, 1994a). An earlier investigation of the same group of workers reported that DNA single-strand breaks — measured by the DNA unwinding method — were also induced in workers estimated to have higher exposure to styrene (average, 300 mg/m^3) calculated from post-shift urinary mandelic acid and blood concentrations of styrene glycol (Mäki-Paakkanen *et al*., 1991). Lack of persistence of the DNA damage indicated that the strand breaks were repaired in the exposed workers. Three studies reported on DNA breakage, measured by use of the single-cell gel electrophoresis assay, in lymphocytes of lamination workers exposed to styrene in the reinforced-plastics industry (exposure range, 68–101 mg/m^3) (Vodicka *et al.*, 1995; Somorovská *et al.*, 1999; Vodicka *et al.*, 1999). Smoking habits had no effect on the level of styrene-induced DNA damage. One study

reported a significant correlation between DNA single-strand breaks and O^6-dG-styrene adduct levels (Vodicka *et al*., 1995).

(iii) *Gene mutations and cytogenetic damage*

Since the previous evaluation of styrene, results from three studies of styrene-induced gene mutations in lymphocytes from reinforced-plastics workers have been published. One study assessed glycophorin A (GPA) variant frequency in erythrocytes of 47 workers exposed for 8 h per day to a time-weighted average concentration of 37 ppm [158 mg/m^3] styrene in the breathing zone. Overall there was no significant increase in variant frequency for GPA allele duplication or allele loss, but a significant increase was found for 28 workers with exposure ≥ 85 mg/m^3 styrene (Bigbee *et al*., 1996). Studies that investigated mutation induction at the *HPRT* locus in lymphocytes of styrene-exposed lamination workers in two different plants reported inconclusive to weak positive results (Vodicka *et al.*, 1995, 1999). Both studies reported a higher mutation frequency in exposed workers than in controls, but the increase was significant in only one of the studies (21.5 ± 9.96 versus 12.69 ± 4.56 mutants per 10^6 cells; $p = 0.039$) (Vodicka *et al.*, 1999).

The previous review of cytogenetic damage in workers occupationally exposed to styrene (IARC, 1994a) showed mainly negative results for the induction of chromosomal aberrations, micronuclei or sister chromatid exchange. Occupational exposure to styrene induced chromosomal aberrations in nine of 22 studies, micronuclei in three of 11 studies and sister chromatid exchange in three of 12 studies. The mean styrene concentration in these studies ranged from 11 to 138 ppm [47–588 mg/m^3]. Workers having no significant increase in chromosomal aberrations were exposed to styrene at concentrations ranging from 2 to 70 ppm [8.5–298 mg/m^3] (IARC, 1994a). The results from six recent cytogenetic studies of styrene-exposed workers in the plastics and boat-manufacturing industries are summarized in Table 12; chromosomal aberrations and sister chromatid exchange were increased in three of three and three of four studies, respectively, while all three studies on micronuclei were negative. The ranges of styrene concentrations to which workers were exposed are also given in Table 12.

Artuso *et al*. (1995) reported that chromosomal aberrations and sister chromatid exchange were induced in lymphocytes of boat-manufacturing workers in both a high- (20–331 ppm [85–1410 mg/m^3]) and low- (0.5–2.9 ppm [2.1–12.4 mg/m^3]) exposure group. The response in the low-exposure group, however, was weak. Workers in a glass fibre-reinforced plastics facility who were also exposed to low concentrations of styrene (0.5–26 ppm [2.1–111 mg/m^3]) did not show increased sister chromatid exchange nor increased induction of micronuclei (Van Hummelen *et al*., 1994). One further study reported that sister chromatid exchange frequencies were significantly increased in lymphocytes of furniture manufacturers who were exposed to styrene from polyester resins at concentrations (20–300 ppm [85–1280 mg/m^3]), similar to those reported in reinforced-plastics facilities (Karakaya *et al*., 1997).

Table 12. Cytogenetic studies in lymphocytes from individuals occupationally exposed to styrene

No. of exposed	No. of controls	Years of exposure		Styrene in air (mg/m^3)		Urinary mandelic acid (mg/g creatinine)		Cytogenetic observation			Reference
		Range	Mean	Range	Mean	Range	Mean	CA	MN	SCE	
28	20	1–26	8	125–180	157[a]		652 mg/L			+	Hallier *et al*. (1994)
52	24		2.9	2.2–111	31	11–649	102		–	–	Van Hummelen *et al*. (1994)
18	18	10–22	14.3	NR			328	+	–		Anwar & Shamy (1995)
23	51			86–1390 2–120				+ (+)		+ (+)	Artuso *et al*. (1995)
53	41		9.9	85–1280	129	14–1482[b]	207[b]		–	+	Karakaya *et al*. (1997)
44	19		14	27–199[c]	101			+			Somorovská *et al*. (1999)

CA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchange; NR, not reported

[a] After modifications were made to the plant to lower styrene concentrations below 85 mg/m^3, results from SCE test were negative.

[b] Mandelic acid + phenylglyoxylic acid

[c] Range of means

(iv) *Protein adducts*

Christakopoulos *et al*. (1993) compared 17 styrene-exposed reinforced-plastics workers (years of exposure: 0.2–15; mean, 6.7) with 11 non-exposed controls and found linear correlations between concentrations of haemoglobin adducts and free styrene 7,8-oxide in blood (r = 0.71), styrene glycol in blood (r = 0.94) and mandelic acid in urine (r = 0.92). [The Working Group noted that no information was available on styrene exposure concentrations for these workers.] Severi *et al.* (1994) looked for haemoglobin adducts of styrene 7,8-oxide in 52 workers having an average styrene exposure of 31 mg/m^3 and found none (detection limit of 10 pmol/g).

Yeowell-O'Connell *et al*. (1996) measured adducts in 48 workers exposed to both styrene (0.9–235 mg/m^3; mean, 64.3 mg/m^3) and styrene 7,8-oxide (13.4–525 µg/m^3; mean, 159 µg/m^3 for 20 subjects) in a boat-building plant. They found no increase in the amount of haemoglobin adducts, but levels of albumin adducts increased with exposures to both styrene and styrene 7,8-oxide. Further analysis of these data (Rappaport *et al*., 1996) indicated that the albumin adducts correlated better with airborne styrene 7,8-oxide than with styrene in those individuals for whom exposure to both chemicals was measured. Fustinoni *et al*. (1998) compared 22 workers selected on the basis of high levels of exposure to styrene (estimated to be about 100 mg/m^3) with 15 controls. The mean levels of 2-phenylethanol and 1-phenylethanol — obtained by chemical cleavage of styrene 7,8-oxide–cysteine adducts — were respectively 2.84 and 0.60 nmol/g albumin and 5.44 and 0.43 nmol/g haemoglobin for the workers and 2.74 and 0.50 nmol/g albumin and 5.27 and 0.39 nmol/g haemoglobin for controls. Differences between workers and controls were significant only when the data for the workers were stratified for high exposure. Johanson *et al*. (2000) exposed four male volunteers to 50 ppm [213 mg/m^3] [$^{13}C_8$]styrene for 2 h. Maximal concentrations of styrene 7,8-oxide in blood (average, 6.7 nM) were seen at 2 h. Hydroxyphenylethylvaline was estimated at 0.3 pmol/g globin. The major (> 95%) portion of the [$^{13}C_8$]styrene metabolites in urine was derived from hydrolysis of styrene 7,8-oxide, with less than 5% coming from metabolism via the phenylacetaldehyde pathway.

(*b*) *Experimental systems* (see Table 13 for references)

Results from studies published since the previous evaluation of styrene are summarized in Table 13.

(i) *DNA adducts*

Both *N*7- and *O*^{6}-deoxyguanosine adducts were formed in NMRI mouse liver, lung and spleen evaluated 3 h after a single intraperitoneal injection of styrene at doses ranging from 0.28 to 4.35 mmol/kg bw (Pauwels *et al*., 1996). A dose–response relationship was reported for both adducts in all three tissues. The *N*7-alkyldeoxyguanosine was more abundant than the *O*^{6}-deoxyguanosine adduct and the levels of both adducts were highest in lung tissue. Samples of liver and lung DNA, taken from CD-1 mice and Sprague-Dawley rats 42 h after a 6-h exposure to 160 ppm [682 mg/m^3] [^{14}C]styrene,

Table 13. Genetic and related effects of styrene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, somatic mutation (*w/w+* mosaic assay)	–[c]		1040 µg/mL, feed	Rodriguez-Arnaiz (1998)
Sister chromatid exchange, human lymphocytes *in vitro*	+		1	Chakrabarti *et al.* (1993)
Sister chromatid exchange, human lymphocytes *in vitro*	+		52	Lee & Norppa (1995)
DNA strand breaks (single-cell gel electrophoresis assay), C57BL/6 mouse liver, kidney, bone marrow and lymphocytes *in vivo*	+[d]		250 ip × 1	Vaghef & Hellman (1998)
Sister chromatid exchange, Fischer 344 rat lymphocytes *in vivo*	–		4260 mg/m^3 (inh.), 6 h/d, 5 d/w, 4 w	Preston & Abernethy (1993)
Chromosomal aberrations, Fischer 344 rat lymphocytes *in vivo*	–		4260 mg/m^3 (inh.), 6 h/d, 5 d/w, 4 w	Preston & Abernethy (1993)
DNA adducts in NMRI mouse liver, lung, spleen and blood, *in vivo*	+		30 ip × 1	Pauwels *et al.* (1996)
DNA adducts in Sprague Dawley rat liver and lung, *in vivo*[e]	+		682 mg/m^3 (inh.), 6 h	Boogaard *et al.* (2000b)
DNA adducts in CD-1 mouse liver and lung, *in vivo*[f]	+		682 mg/m^3 (inh.), 6 h	Boogaard *et al.* (2000b)
DNA adducts (O^6-guanine) (^{32}P-postlabelling) in female CRL CD-1 mouse lung *in vivo*	–		682 mg/m^3 (inh.), 6 h/d, 5 d/w, 2 w	Otteneder *et al.* (2002)
DNA adducts (O^6-guanine) (^{32}P-postlabelling) in female CRL CD rat lung *in vivo*	–		2130 mg/m^3 (inh.), 6 h/d, 5 d/w, 2 w	Otteneder *et al.* (2002)
DNA adducts (O^6-guanine) (^{32}P-postlabelling) in male and female CRL CD rat liver *in vivo*	+		4260 mg/m^3 (inh.), 6 h/d, 5 d/w, 2 y	Otteneder *et al.* (2002)
Chromosomal aberrations, human lymphocytes *in vivo*	?		27 mg/m^3	Somarovská *et al.* (1999)

[a] +, positive;–, negative

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day (unless otherwise specified); d, day; ip, intraperitoneal; inh., inhalation; w, week; y, year; h, hour

[c] A positive response was seen at this dose in insecticide-resistant strains, which have high bioactivation capacities.

[d] Positive response in bone marrow at 350 mg/kg bw

[e] DNA adduct level was 3-fold higher in lung Type-II cells than in total lung.

[f] DNA adduct levels in Clara and non-Clara cells were similar to that in total lung.

showed increased levels of *N*7-deoxyguanosine adducts for both species (Boogaard *et al*., 2000b). The adduct level in rat lung fractions enriched in type II cells was approximately three times higher than that in total lung, whereas the adduct level in mouse lung fractions enriched in Clara cells was similar to that of total lung. In these studies, the covalent binding indices (CBI) were all below 0.5.

Otteneder *et al*. (2002) did not detect O^6-deoxyguanosine adducts in DNA from lung tissue of CD-1 mice or CD rats exposed to 160 ppm or 500 ppm [682 or 2130 mg/m^3] styrene, respectively for two weeks (6 h per day, five days per week). The limit of detection in the ^{32}P-postlabelling method used in this study was one adduct per 10^7 nucleotides. However, both α and β isomers of O^6-dG-styrene 7,8-oxide adducts were detected in DNA from CD rats that were exposed by inhalation to 1000 ppm [4260 mg/m^3] styrene for two years (6 h per day, five days per week). The authors noted that DNA adduct formation in tissue homogenates did not reflect either the species difference in carcinogenicity nor the target organ specificity of styrene.

(ii) *Mutation and allied effects*

Positive and negative results have previously been reported for styrene-induced mutations in *Salmonella typhimurium* (IARC, 1994a). Positive results were reported in studies using strains TA1530 or TA1535 with the addition of an exogenous metabolic activation system. No new data on mutations in *S. typhimurium* have been reported.

Results from one study of somatic mutations in *Drosophila melanogaster* were negative. This study used both insecticide-susceptible (IS) and -resistant (IR) strains, which differ in their activities of drug-metabolizing enzymes, to evaluate mutations in the *w/w+* mosaic assay. Females were exposed to styrene at a concentration of 1040 μg/mL in the feed medium. Although negative results were reported for the IS strain, positive results were seen in the IR strain at this dose. Two studies of the induction of sister chromatid exchange in human lymphocytes exposed to styrene *in vitro* also gave positive results. These observations are consistent with those from earlier studies.

DNA strand breaks were induced in liver, kidney, bone marrow and lymphocytes of C57BL/6 mice given a single intraperitoneal injection of styrene. On the other hand, neither chromosomal aberrations nor sister chromatid exchange were seen in lymphocytes of Fischer 344 rats exposed to styrene by inhalation at a concentration of 1000 ppm [4260 mg/m^3] for 6 h per day on five days per week for four weeks.

4.4.2 *Styrene 7,8-oxide*

(*a*) *Humans*

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

(*b*) *Experimental systems* (see Table 14 for references)

(i) *DNA adducts*

A detailed description of *N*7, N^2 and O^6-deoxyguanosine adducts of styrene 7,8-oxide formed *in vivo* and *in vitro* was previously presented (IARC, 1994b). Subsequent studies have reported that *N*7-deoxyguanosine adducts are formed in human whole blood and embryonic lung fibroblasts *in vitro*, as well as in salmon testis DNA, upon exposure to styrene 7,8-oxide. In addition, O^6-deoxyguanosine adducts were induced in human lymphocytes exposed to 24 µg/mL styrene 7,8-oxide *in vitro* and in rat liver DNA following inhalation exposure to 1000 ppm [4.9 mg/m^3] styrene 7,8-oxide for 6 h per day on five days per week for 104 weeks. DNA adducts were not clearly induced in other studies in which rats were exposed to styrene 7,8-oxide by intraperitoneal injection or oral exposure.

(ii) *Mutation and allied effects*

Styrene 7,8-oxide, a monofunctional alkylating agent, has given positive results in virtually every short-term genetic assay *in vitro* and, to a lesser degree, *in vivo*. Positive results have previously been reported for gene mutations in bacteria and mammalian cells *in vitro*. DNA strand breaks, alkali-labile sites, and cytogenetic damage also have been reported in mammalian cells *in vitro* and, to some degree, *in vivo*.

It was noted in the previous evaluation (IARC, 1994b) that the *R*-enantiomer of styrene 7,8-oxide may be slightly more mutagenic in *S. typhimurium* TA100 than the *S*-enantiomer (Pagano *et al*., 1982; Seiler, 1990; Sinsheimer *et al*., 1993). In contrast, Sinsheimer *et al*. (1993) reported that the *S*- but not the *R*-enantiomer induced chromosomal aberrations and sister chromatid exchange in mouse bone marrow.

[The Working Group noted that, except when the *R*- or *S*- enantiomer of styrene 7,8-oxide was specifically tested, exposures to styrene 7,8-oxide were probably mixtures of optical isomers.]

Results from studies on genetic and related effects of styrene 7,8-oxide published since the earlier evaluation are essentially all positive. In fact, only one study reported negative results, the *w/w+* mosaic assay for induction of somatic mutations in insecticide-sensitive *Drosophila melanogaster*. However, in insecticide-resistant *Drosophila* strains, which have an active bioactivation system, styrene 7,8-oxide induced somatic mutations.

DNA single-strand breaks and alkali-labile sites were induced in Wistar rat and human testicular and Chinese hamster V-79 cells *in vitro*. Four studies reported that DNA strand breaks and/or fragmentation were induced in human lymphocytes and human embryonic lung fibroblasts exposed to styrene 7,8-oxide *in vitro*. Similarly, positive results were reported for the induction of gene mutations at the *HPRT* locus in human lymphocytes and B-cell lymphoblastoid cells treated *in vitro*. Six studies reported that exposure to styrene 7,8-oxide *in vitro* induced a significant increase in sister chromatid exchange frequencies in human lymphocytes. One study also reported a significant

Table 14. Genetic and related effects of styrene 7,8-oxide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, somatic mutation (*w/w+* mosaic assay)	–		1200 µg/mL[c] in feed	Rodriguez-Arnaiz (1998)
DNA single-strand breaks and alkali-labile sites (alkaline elution), Wistar rat testicular cells *in vitro*	+	NT	12	Bjørge *et al.* (1996)
DNA single-strand breaks and alkali-labile sites (alkaline elution), Chinese hamster V-79 cells, *in vitro*	+	NT	6	Herrero *et al.* (1997)
DNA strand breaks, human lymphocytes *in vitro*	+	NT	6	Bastlová *et al.* (1995)
DNA single-strand breaks and alkali-labile sites (alkaline elution), human testicular cells *in vitro*	+	NT	12	Bjørge *et al.* (1996)
DNA single-strand breaks human embryonic lung fibroblasts *in vitro*	+	NT	1.2	Vodicka *et al.* (1996)
DNA fragmentation, human whole blood *in vitro*	+	NT	1	Marczynski *et al.* (1997a)
DNA damage (alkaline single-cell gel electrophoresis assay), human lymphocytes *in vitro*	+	NT	2.4	Laffon *et al.* (2001)
Gene mutation, human lymphocytes, *HPRT* locus *in vitro*	(+)	NT	24	Bastlová *et al.* (1995)
Gene mutation, human T-lymphocytes, *HPRT* locus, *in vitro*	+	NT	24	Bastlová & Podlutsky (1996)
Gene mutation, human B-cell lymphoblastoid cells, *HPRT* locus, *in vitro*	(+)[d]	NT	72	Shield & Sanderson (2001)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	6	Lee & Norppa (1995)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	6	Ollikainen *et al.* (1998)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	6	Uusküla *et al.* (1995)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	6	Laffon *et al.* (2001)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	12	Chakrabarti *et al.* (1997)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	12	Zhang *et al.* (1993)
Micronucleus test, human lymphocytes *in vitro*	+	NT	12	Laffon *et al.* (2001)
DNA alkali-labile sites (alkaline single-cell gel electrophoresis assay), C57BL/6 mouse liver, kidney, bone marrow and lymphocytes *in vivo*	+		100 ip × 1	Vaghef & Hellman (1998)
DNA alkali-labile sites (alkaline single-cell gel electrophoresis assay), ddY mouse organs *in vivo*	+		400 ip × 1	Tsuda *et al.* (2000)
DNA alkali-labile sites (alkaline single-cell gel electrophoresis assay), CD-1 mouse organs *in vivo*	+		400 ip × 1	Sasaki *et al.* (1997)
DNA adducts (*N*7-guanine), salmon testis DNA *in vitro*	+	NT	11	Koskinen *et al*. (2000)
DNA adducts (*N*7-guanine) in human embryonic lung fibroblasts *in vitro*	+	NT	1.2	Vodicka *et al.* (1996)
DNA adducts (*N*7-guanine) in human whole blood, *in vitro*	+	NT	NR	Pauwels & Veulemans (1998)
DNA adducts (O^6-guanine) in human lymphocytes *in vitro*	(+)	NT	24	Bastlová *et al.* (1995)
DNA adducts (O^6-guanine), rat liver *in vivo*	+		4260 mg/m^3 (inh.), 6 h/d, 5 d/w, 104 w	Otteneder *et al.* (1999)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; NR, not reported

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; d, day; ip, intraperitoneal; inh., inhalation

[c] Positive results reported in insecticide-resistant (IR) strains at 600 µg/mL (5mM)

[d] GSTM1-null more sensitive

increase in the induction of micronuclei in human lymphocytes treated *in vitro*. These results are consistent with those previously reviewed.

Three recent studies have reported that DNA strand breaks and alkali-labile sites are induced in multiple tissues (lung, liver, kidney, bone marrow and lymphocytes) of mice exposed to styrene 7,8-oxide by a single intraperitoneal injection at doses of 100–400 mg/kg bw. Results from previously evaluated studies of induction of cytogenetic damage in rodents exposed to styrene 7,8-oxide *in vivo* were predominately negative. Two of three studies reported some evidence of exposure-related sister chromatid exchange induction and two of four studies reported that styrene 7,8-oxide induced chromosomal aberrations. Neither of the two micronucleus studies previously evaluated reported increases in the frequency of micronuclei following exposures to styrene 7,8-oxide via intraperitoneal injection (IARC, 1994b).

4.5 Mechanistic considerations

Following chronic exposure to styrene, a carcinogenic response has been observed only in mouse lung, but not in tissues of rats.

Studies in humans and experimental animals and with tissues from both rodents and humans *in vitro* using a variety of experimental approaches have attempted to determine the mode of action for the styrene toxicity and carcinogenicity observed in experimental animals. In general, the objectives of many of these studies have been (1) to explore the basis for the observed interspecies differences in tumour response between rats and mice, (2) to determine the critical steps in styrene carcinogenicity and (3) to identify the most likely chemical species responsible for the initiation of tumour development.

At least two plausible modes of action, which are not mutually exclusive, for styrene carcinogenicity are suggested by the existing data. One involves a DNA-reactive mode of action initiated by the metabolic conversion of styrene to styrene 7,8-oxide, a genotoxic metabolite of styrene, and the subsequent induction of DNA damage in target tissues; the other involves cytotoxic effects in lungs of mice exposed to styrene. For either mode of action, interspecies differences in the metabolism and toxicokinetics of styrene and styrene 7,8-oxide that exist between rats and mice are likely to play a key role.

4.5.1 *Interspecies differences in toxicokinetics and metabolism*

A number of studies in human volunteers and in workers occupationally exposed to styrene by inhalation (summarized by Bond, 1989) have shown that styrene toxicokinetics and metabolic pathways are qualitatively similar in humans and experimental animals, although there are quantitative differences. In humans, between 60 and 70% of inhaled styrene is absorbed and more than 85% of the absorbed styrene is eliminated in the urine as mandelic and phenylglyoxylic acids. These metabolites are formed following hydrolysis of styrene 7,8-oxide, which indicates that humans metabolize styrene to

styrene 7,8-oxide. Johanson *et al*. (2000) have quantified blood levels of styrene 7,8-oxide in human volunteers exposed to styrene.

In all experimental animal species studied, styrene is rapidly metabolized to styrene 7,8-oxide following absorption by oral, dermal or inhalation exposure. Styrene 7,8-oxide is sufficiently stable to be readily detected in the blood of both rats and mice exposed to styrene. Systemic styrene 7,8-oxide concentrations do not, however, explain interspecies differences in the carcinogenic response to styrene. At styrene exposure concentrations of 1000 ppm [4260 mg/m^3] (the highest concentration tested), styrene 7,8-oxide concentrations in the blood of rats were two orders of magnitude higher than those in the blood of mice exposed to 20–40 ppm [85–170 mg/m^3] styrene (the lowest concentrations causing tumours), although mice, but not rats, develop lung tumours (Cruzan *et al*., 1998, 2001).

The enzymatic oxidation of styrene to styrene 7,8-oxide is mediated by several isoforms of cytochrome P450 (CYP) enzymes including CYP2E1, CYP2B6 and CYP2F1 in humans. In experimental animals, CYP2E1 appears to be the major isoform responsible for styrene oxidation in the liver, and CYP2F2 is the major form in the mouse lung, as determined by in-vitro studies (Carlson, 1997a). Interspecies differences exist in the rates of metabolism of styrene by liver and lung tissue. In particular, human lung tissue produces less styrene 7,8-oxide than that of rats and considerably less than that of mice (Nakajima *et al*., 1994a; Carlson *et al*., 2000; Filser *et al.*, 2002).

The metabolism of styrene to styrene 7,8-oxide in mouse lung occurs almost exclusively in the Clara cells and the rate of styrene oxidation in these cells is threefold faster than in Clara cells of rats (Hynes *et al*., 1999). The mouse Clara cell may be at greater risk than the rat Clara cell if locally generated, rather than extrapulmonary, styrene 7,8-oxide is critical for cytotoxic or genotoxic damage.

Metabolism of styrene 7,8-oxide can proceed through hydrolysis of styrene 7,8-oxide by epoxide hydrolase or through conjugation with glutathione mediated by glutathione *S*-transferase. Conversion to phenylacetaldehyde and other ring-opened metabolites provides additional pathways for styrene and styrene 7,8-oxide metabolism (Sumner & Fennell, 1994; Johanson *et al.*, 2000). Stable products representing each of these pathways are eliminated in the urine. Interspecies differences exist in the proportion of styrene 7,8-oxide that is eliminated via each of these pathways, and these differences may play a role in the species sensitivity towards the toxic and carcinogenic effects of styrene exposure. For example, the capacity of epoxide hydrolase in human tissues to detoxify styrene 7,8-oxide exceeds that of rat or mouse tissues. In contrast, the activity of glutathione *S*-transferase in human tissues is much lower than in rodent tissues (summarized in Cohen *et al.*, 2002). Mouse tissues show significantly higher rates of activation of styrene to DNA-reactive epoxides than do rat or human tissues. While mouse, rat and human tissues all use epoxide hydrolase to detoxify styrene 7,8-oxide, the mouse also uses glutathione *S*-transferase to a significant extent, with less activity in the rat and very little in humans.

The role of glutathione in the detoxication of styrene 7,8-oxide introduces the possibility that high concentrations of styrene 7,8-oxide significantly deplete the cellular concentrations of glutathione, resulting in substantial cellular damage. Significant reductions in pulmonary glutathione were noted in mice exposed repeatedly to 80–300 ppm [341–1280 mg/m^3] of styrene (Filser *et al*., 2002). Rats were significantly less susceptible to glutathione depletion than mice. However, while glutathione depletion in mouse lung has not been demonstrated at styrene concentrations below 80 ppm [341 mg/m^3], evidence of cancer induction exists at exposure concentrations as low as 20 ppm [85 mg/m^3].

In summary, quantitative, but not qualitative, differences exist in the toxicokinetics of styrene and styrene 7,8-oxide in mice, rats and humans. These quantitative differences alone do not appear to be sufficient to account for the development of lung tumours in mice at low styrene exposures and the resistance of rats to tumour formation at high exposures. Thus, factors of a toxicodynamic nature may play a role in the interspecies differences in response to styrene.

4.5.2 *Interspecies differences in toxicodynamics and mode of action*

In general, mice appear to be more susceptible to lung tumour induction by epoxides and epoxide-forming chemicals than rats (Melnick & Sills, 2001). Inhalation of ethylene oxide, 1,3-butadiene, isoprene and chloroprene induced lung tumours in mice but not rats (Lynch *et al*., 1984; National Toxicology Program, 1984; Snellings *et al*., 1984; National Toxicology Program, 1987; Owen *et al*., 1987; Melnick *et al*., 1994; National Toxicology Program, 1998, 1999; Melnick & Sills, 2001). The determinants underlying the susceptibility of the mouse lung towards tumour formation may rely in part on toxicokinetic considerations, but toxicodynamic determinants are probably also at play.

One potential mode of action for styrene involves the cytotoxicity of styrene 7,8-oxide. Repeated exposure to styrene progressively results in focal crowding of bronchiolar cells, bronchiolar epithelial hyperplasia and bronchiolo-alveolar hyperplasia in the lungs of mice but not in those of rats (Cruzan *et al*., 1997, 1998; Cohen *et al*., 2002; Cruzan *et al*., 2001). A major factor that may play a role in the onset of hyperplasia in mice after exposure to styrene is pulmonary formation of styrene 7,8-oxide. Styrene 7,8-oxide administered to mice by intraperitoneal injection results in pulmonary toxicity as measured by release of enzymes into bronchoalveolar lavage fluid, suggesting that styrene 7,8-oxide is cytotoxic to mouse lung cells. This cytotoxicity stimulates cell replication and proliferation. The pulmonary toxicity induced by high doses of injected styrene 7,8-oxide also clearly demonstrates that systemically available styrene 7,8-oxide (i.e., that produced in extrapulmonary tissues and released into the circulation) can enter lung cells to induce a toxic response.

A second mode of action for styrene-induced carcinogenicity invokes DNA-reactivity and subsequent genotoxicity. Although styrene itself is not DNA-reactive, styrene 7,8-oxide binds covalently to macromolecules including proteins and nucleic acids.

Styrene 7,8-oxide can bind to DNA with the formation of stable N^2 and O^6 adducts of deoxyguanosine in human lymphocytes and cultured mammalian cells (Horvath *et al*., 1994; Bastlová *et al*., 1995; Vodicka *et al*., 1999). In particular, the O^6 adducts are relatively persistent. The stability of the O^6 adducts is supported by studies of styrene 7,8-oxide adducts in the lymphocytes of workers exposed to styrene (Vodicka *et al*., 1994, 1995, 1999).

The genotoxicity of styrene and styrene 7,8-oxide has been studied extensively in both experimental systems and humans. Styrene 7,8-oxide has been shown to cause mutations (with and without metabolic activation) in the Ames *Salmonella* assay. In contrast, styrene generally gives negative results for mutagenic activity in the Ames *Salmonella* assay; however, it was noted previously (IARC, 1994a) that positive results in *Salmonella typhimurium* were seen in some studies in the presence of exogenous metabolic activation. The evidence for mutations in humans occupationally exposed to styrene is equivocal.

Exposure to styrene results in an increase in sister chromatid exchange frequency and, to a limited extent, the frequency of chromosomal aberrations and micronucleated cells in experimental animals. Cytogenetic studies in exposed workers are difficult to interpret due to potential confounders and co-exposures to other genotoxic agents (Scott & Preston, 1994). Assessing quantitative relationships across human studies has shown associations between styrene exposure and the frequency of chromosomal abnormalities but not sister chromatid exchange or micronuclei (Bonassi *et al*., 1996; Cohen *et al*., 2002). Rappaport *et al*. (1996) reported a positive correlation between airborne styrene 7,8-oxide concentrations, but not styrene concentrations, and sister chromatid exchange frequency in styrene workers.

An association has been reported between styrene exposure and DNA strand breaks in workers employed in various styrene-related industries (Mäki-Paakkanen *et al*., 1991; Somorovská *et al*., 1999). However, the role of these strand breaks in disease etiology has been questioned since this type of damage is quickly and efficiently repaired in both animals and humans (Walles *et al*., 1993; Bastlová *et al*., 1995). As an example, studies by Walles *et al*. (1993) indicate that end-of-shift DNA damage in subjects occupationally exposed to styrene is repaired by the next morning. The DNA strand breaks are probably caused by the much less persistent *N*7 adducts (Vodicka *et al*., 1996).

4.5.3 *Conclusion*

In summary, data from both laboratory (*in vitro* and *in vivo*) and human studies indicate that styrene exposure can result in low levels of DNA adducts and DNA damage in individuals who possess the capacity to activate styrene metabolically to its epoxide metabolite, styrene 7,8-oxide. However, as noted above, mice, but not rats, develop lung tumours following styrene exposure, even though both species form DNA adducts. DNA adducts are also found in organs other than the lung.

Circulating styrene 7,8-oxide may also play a role. However, the concentration of this metabolite in rat blood is two orders of magnitude higher than in the mouse. The lung tumours in mice probably develop as a result of in-situ formation of styrene 7,8-oxide which leads to cytotoxicity and increased cell proliferation, but a role of circulating styrene 7,8-oxide and of DNA adducts cannot be discounted.

Based on metabolic considerations, it is likely that the proposed mechanism involving metabolism of styrene to styrene 7,8-oxide in mouse Clara cells is not operative in human lungs to a biologically significant extent. However, based on the observations in human workers regarding blood styrene 7,8-oxide, DNA adducts and chromosomal damage, it cannot be excluded that this and other mechanisms are important for other organs.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Styrene is a commercially important monomer which is used extensively in the manufacture of polystyrene resins (plastic packaging, disposable cups and containers, insulation) and in copolymers with acrylonitrile and/or 1,3-butadiene (synthetic rubber and latex, reinforced plastics). Human exposure occurs at levels of milligrams per day during its production and industrial use and at much higher levels in the glass fibre-reinforced plastics industry. Exposure to the general population occurs at levels of micrograms per day due mainly to inhalation of ambient air and cigarette smoke and intake of food that has been in contact with styrene-containing polymers.

5.2 Human carcinogenicity data

Retrospective cohort studies of styrene have been conducted in three types of industry: production of styrene monomer and styrene polymers, production of glass fibre-reinforced plastic products, and production of styrene–butadiene rubber.

In a European multinational cohort study of workers in the glass fibre-reinforced plastics industry (the largest component of which was Danish), there was no excess mortality from lymphatic and haematopoietic neoplasms in the entire cohort in comparison with the general population, but the results may have been biased by problems with mortality ascertainment in some of the sub-cohorts. In internal analyses, using unexposed cohort members as the comparison group, the risk of lymphatic and haematopoietic neoplasms was significantly increased among exposed workers after more than 20 years since their first exposure to styrene, and increased with increasing intensity of exposure but not with increasing cumulative exposure to styrene.

Another study of cancer incidence in the reinforced-plastics industry conducted in Denmark involved many workers who had been included in the European study. Overall,

a small and non-significant excess of leukaemia was observed. A significant excess of leukaemia was observed among workers employed before 1971 (the period with the highest styrene exposures). There was also a significant increase in the incidence of leukaemia when attention was restricted to the follow-up period after 10 years since first exposure to styrene, but only among workers with very short employment (less than 1 year). There was evidence that the available data on duration underestimated the true duration of exposure for many of the workers. (This also applies to the European cohort since the Danish cohort constituted a large fraction of this cohort).

A large study of workers exposed to styrene in the reinforced-plastics industry in the USA found no overall excess of lymphatic and haematopoietic neoplasms.

Two studies of chemical workers in the USA and the United Kingdom involved in the production of styrene and styrene derivatives found a weak association between exposure to styrene and lymphatic and haematopoietic cancers. Styrene exposures were poorly documented in these studies, and exposures to several other chemicals may have occurred.

A follow-up study of cancer incidence in Finnish workers biologically monitored for occupational exposures to styrene during the 1970s and early 1980s did not show any increase in risk for lymphatic and haematopoietic neoplasms. The relatively small size of this study and the low exposures of workers detracted from its power to detect an effect of the magnitude found in some of the other studies.

A small excess of leukaemia mortality has been reported in studies of styrene–butadiene workers in the USA. This excess increased with cumulative exposure to styrene in analyses that only considered this exposure; however, in analyses that included 1,3-butadiene, the exposure–response relationship became non-monotonic. Interpretation of the findings from this study is hampered by the high correlation between styrene and 1,3-butadiene exposures, which makes it difficult to disentangle the effects of these two exposures.

There have also been reports of increased risks of rectal, pancreatic and nervous system cancers in some of the cohort and case–control studies. The numbers of cases were quite small in these studies, and most of the larger cohort studies have not yielded similar findings. Many of the cohort studies did not examine these sites in detail.

The studies of glass fibre-reinforced plastics workers are the most informative with regard to the hypothesis that styrene exposure is associated with an increased risk of cancer in humans. This is because these workers had higher styrene exposures and less potential for exposure to other substances than the other cohorts studied. On the other hand, they are hampered by the high mobility of this workforce. In the overlapping European and Danish studies, a small excess of lymphatic and haematopoietic neoplasms was found, particularly in subgroup analyses of workers with relatively high exposures and a sufficiently long time (e.g., > 10 years) since first exposure. There was no relationship between lymphatic and haematopoietic neoplasms and cumulative styrene exposure, but these studies had problems in accurately estimating duration of employment and hence cumulative exposures.

The increased risks for lymphatic and haematopoietic neoplasms observed in some of the studies are generally small, statistically unstable and often based on subgroup analyses. These findings are not very robust and the possibility that the observations are the results of chance, bias or confounding by other occupational exposures cannot be ruled out.

5.3 Animal carcinogenicity data

Styrene was tested for carcinogenicity in mice in one inhalation study and four oral gavage studies. In the inhalation study, in male mice there was an increase in the incidence of pulmonary adenomas and in female mice, there was an increase in the incidence of pulmonary adenomas, and only an increase in that of carcinomas in the high-dose group. Two of the gavage studies were negative. The other two were considered inadequate for an evaluation of the carcinogenicity of styrene. A screening study by intraperitoneal administration also did not find an increase in tumour incidence or multiplicity in mice.

Styrene was tested for carcinogenicity in rats in four gavage studies, one drinking-water study and two inhalation studies. Overall, there was no reliable evidence for an increase in tumour incidence in rats.

Styrene 7,8-oxide is a major metabolite of styrene and has been evaluated previously (IARC, 1994b). The evaluation at that time was that there was *sufficient evidence* in experimental animals for the carcinogenicity of styrene 7,8-oxide.

5.4 Other relevant data

Styrene is absorbed following exposure via inhalation, dermal contact and orally in humans and laboratory animals. In humans, approximately 70% of the inhaled dose is absorbed. Styrene is distributed throughout the body, with the highest concentration generally found in adipose tissue. There are both quantitative and qualitative interspecies differences in styrene metabolism. In humans, styrene is metabolized primarily via the styrene 7,8-oxide pathway to be excreted in the urine as mandelic and phenylglyoxylic acids. In rodents, but not in humans, glutathione conjugation of styrene 7,8-oxide to form mercapturic acids is an important metabolic pathway. Metabolism of styrene to 1- and 2-phenylethanol and then to phenylacetaldehyde and finally to phenylacetic, phenylaceturic and hippuric acids is more important in animals than in humans.

CYP2E1 and CYP2F are the most important cytochrome P450 enzymes in rodents and humans responsible for the metabolism of styrene to styrene 7,8-oxide. In addition, CYP2B6 may be important in humans. *In vitro*, the rates of metabolism of styrene to styrene 7,8-oxide are much higher in mouse lung than in rat or human lung.

Occupational styrene exposure causes central and peripheral nervous system effects in humans. It causes a reversible decrease in colour discrimination and in some studies effects on hearing have been reported. Studies of effects of styrene on the haematopoietic

and immune systems, liver and kidney in exposed workers did not reveal consistent changes.

Central nervous system effects of styrene were reported in rats, guinea-pigs and rabbits. Styrene exposure causes liver and lung toxicity in mice and nasal toxicity in rats and mice.

In humans, there is no evidence for an association between workplace exposure to styrene and spontaneous abortions, malformations or decreased male fecundity.

In rats, there is some evidence for reduced sperm count and peripubertal animals may be more sensitive than adult animals. Styrene crosses the placenta in rats and mice. It increases prenatal death at dose levels causing decreased maternal weight gain. Decreased pup weight, postnatal developmental delays as well as neurobehavioural and neurochemical abnormalities have been reported in rats exposed to styrene during pre- or postnatal development. In-vitro studies indicate that the potential for developmental toxicity is much higher for styrene 7,8-oxide than for styrene.

Occupational exposure to styrene leads to formation of O^6-deoxyguanosine (O^6-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′-monophosphate) and *N*7-deoxyguanosine adducts in DNA. Low levels of these two adducts were also detected in liver of mice and rats exposed to styrene.

Inconsistent results have been reported for chromosomal aberrations, micronuclei and sister chromatid exchange in approximately 30 studies of workers exposed to styrene in various industries. These studies were predominantly from the reinforced-plastics industry where styrene exposure is high, but there was no indication of a dose–response relationship in any of the studies reporting positive results. Induction of chromosomal aberrations was reported in 12 of 25 studies, sister chromatid exchange in six of 16 studies and micronuclei in three of 14 studies.

Sister chromatid exchange and to a lesser degree chromosomal aberrations were induced in rodents *in vivo* and consistently in human lymphocytes *in vitro*.

Styrene was predominantly inactive in assays for gene mutations in bacteria, although some studies reported mutations in the presence of a metabolic activation system.

Data from both laboratory (*in vitro* and *in vivo*) and human studies indicate that styrene exposure can result in low levels of DNA adducts and DNA damage in individuals who possess the capacity to activate styrene metabolically to styrene 7,8-oxide. However, as noted above, mice, but not rats, develop lung tumours following styrene exposure, even though both species form DNA adducts. DNA adducts are also found in organs other than the lung. Circulating styrene 7,8-oxide may also play a role. However, the concentration in rat blood is two orders of magnitude higher than in the mouse.

The lung tumours in mice probably develop as a result of in-situ formation of styrene 7,8-oxide which causes cytotoxicity and increased cell proliferation, but the roles of circulating styrene 7,8-oxide and of DNA adducts cannot be discounted. Based on metabolic considerations, it is likely that the proposed mechanism involving metabolism of styrene to styrene 7,8-oxide in mouse Clara cells is not operative in human lungs to a

biologically significant extent. However, based on the observations in human workers regarding blood styrene 7,8-oxide, DNA adducts and chromosomal damage, it cannot be excluded that this and other mechanisms are important for other organs.

5.5 Evaluation[1]

There is *limited evidence* in humans for the carcinogenicity of styrene.
There is *limited evidence* in experimental animals for the carcinogenicity of styrene.

Overall evaluation

Styrene is *possibly carcinogenic to humans (Group 2B)*.

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[1] Two Working Group members, Dr Carlson and Dr Cruzan, recused themselves from the final discussion and the evaluation of styrene.

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