Chapter 5.12 Styrene

General description

Styrene (vinylbenzene, styrole) is a colourless, viscous liquid with a pungent odour and tendency to polymerize. Its chemical structure is $C_6H_5{\cdot}CH{=}CH_2$ and its molecular mass 104.15. Styrene is slightly soluble in water, soluble in ethanol and very soluble in benzene and petroleum ether.

Sources

Styrene is one of the most important monomers worldwide, and its polymers and copolymers are used in an increasingly wide range of applications. The major uses are in plastics, latex paints and coatings, synthetic rubbers, polyesters and styrene-alkyd coatings *(1,2)*.

Among the top 50 chemicals worldwide, styrene was twentieth in 1994 with production of 11 270 million pounds *(3)*. Styrene occurs naturally as a degradation product in cinnamic acid containing plants, e.g. balsamic trees *(4)*, and as a by-product of fungal and microbial metabolism *(5,6)*.

Styrene has been detected in the atmosphere in many locations. Its presence in air is principally due 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 *(7)*.

The concentration of styrene in urban air is relatively low compared with that of aromatic hydrocarbons, such as toluene and xylene. This appears to be due to the ready reactivity of styrene with ozone to yield benzaldehyde and peroxides, all of which are irritants; one of the peroxides, peroxybenzyol nitrate, is a potent eye irritant. Styrene is an active component of photochemical smog.

Some liberation of styrene may also take place from recently manufactured plastic goods. While this may contribute to indoor levels of styrene, the effect on total emissions to the environment is negligible.

Occurrence in air

Styrene emissions reported to the European Union by member countries *(8)* are shown in Table 1. Air emissions in the United States, reported to the US Environmental Protection Agency by industrial facilities, declined from 15 580 tonnes in 1989 to 12 900 tonnes in 1991 *(9)*. In Canada, total reported emissions of styrene from industrial plants in 1993 were estimated to be 1942 tonnes, almost all (1937 tonnes) to air *(10)*. Another Canadian estimate for total annual emission of styrene from industrial activities in that year was 1561 tonnes *(11)*; most of this amount (1000 tonnes) was considered to come from reinforced plastics industry.

Ambient air levels of styrene sampled in the vicinity of seven reinforced plastics processors in three states in the United States ranged from 0.29 to 2934 $\mu g/m^3$, and those in communities

Table 1. Estimated traffic and industrial emissions of styrene in member countries of the European Union (thousand tonnes/year)

Country	Source	
	Road (gasoline)	Chemical industry
Belgium	0.5	0.75
Denmark	0.28	NR[a]
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

[a] NR = not reported.
Source: Bouscaren et al. *(8)*.

near the processors from not detected (<0.15 μg/ m^3) to 23.8 μg/m^3 *(12)*. Except in highly polluted areas, styrene concentrations in outdoor air are generally <1 μg/m^3. In indoor air, e.g. mobile homes, the mean concentrations are frequently somewhat higher (<1–6 μg/m^3), smoking making a significant contribution *(13)*. The styrene content of cigarette smoke has been reported to be 18–48 μg/cigarette *(7)*. Emissions of styrene from some styrene-containing household products may also contribute to indoor air levels *(14)*.

Styrene levels in ambient air were determined in a survey of 18 sites (mostly urban) in Canada in 1988–1990 *(15)*. Altogether 586 24-hour samples were collected and the mean concentrations at the 18 sites 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-hour concentration was <0.48 μg/m^3 (limit of detection); individual values ranged up to 129 μg/m^3 (average, 0.28 μg/m^3) *(15)*.

Thermal degradation of styrene-containing polymers also releases styrene into ambient air *(16)*. Gurman et al. *(17)* reported that styrene monomer is the main volatile product of the thermal decomposition of polystyrene, comprising up to 100% of the volatiles.

Conversion factors

1 ppm = 4.26 mg/m^3
1 mg/m^3 = 0.23 ppm

Routes of exposure

Air

Styrene is present even in unpolluted rural areas in low concentrations. The concentrations in urban atmosphere are around 0.3 μg/m^3, leading to an estimated daily intake of about

6 µg/person *(18)*. In polluted urban air and within 1 km of styrene polymerization units, the concentration can be 20–30 µg/m^3 with an estimated daily intake of 400–600 µg/person living in the area *(18)*. Indoor sources may also contribute to the level of exposure.

In a study carried out in Germany in 1990–1991 with 113 persons selected at random over the country, the geometric mean of personal exposure to styrene was found to be 2.1 µg/m^3. The 95th percentile was 8 µg/m^3 *(19)*.

Occupational 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. Highest exposures have been measured in the reinforced plastics industry *(13)*.

Average exposure of workers to styrene in styrene production and polymerization factories has been reported rarely to exceed 20 ppm (85 mg/m^3), usually due to occasional bursts and leakages of reactors, tubing and other equipment. Surveys conducted in United States plants 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, with occasional peaks of up to 50 ppm *(13)*.

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. Styrene serves as a solvent and a reactant for the unsaturated polyester resin, in which it constitutes about 40% by weight. During lamination and curing, about 10% of the styrene may evaporate into the workplace air *(13)*.

Several factors influence the level of styrene in workplace air. The manufacture of objects with large surface areas, 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 United States 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) *(20)*. In a detailed survey of 12 plants making fibreglass in Washington State, 40% of 8-hour samples contained more than 100 ppm (426 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 *(21)*. In an extensive survey of the reinforced plastics industry in Finland, styrene levels in 77% of respiratory zone measurements for laminators exceeded the hygienic standard of 20 ppm (85 mg/m^3), and the mean 8-hour time-weighted average (TWA) was 43 ppm (range 5–182 ppm) *(22)*.

Environmental tobacco smoke

Cigarette smoking and exposure to environmental tobacco smoke (ETS) may be important sources of styrene exposure. Sidestream smoke from one cigarette releases amounts of styrene ranging from 20 to 48 µg of styrene into the air *(7)*. Wallace et al. *(23)* estimated that the excess concentration of styrene in indoor air in homes with smokers is 0.53 µg/m^3.

Drinking-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. In a survey of Canadian drinking-water supplies, the frequency of detection of styrene was low; when detected, it was generally at a concentration of <1 µg/litre *(15)*.

Styrene has also been detected in drinking-water in the United States at concentrations of less than 1 µg/litre and, specifically, in commercial, charcoal-filtered drinking-water in New Orleans *(18)*.

Styrene evaporates readily from water to air. The evaporation half-time of styrene in water at a depth of 1 m is estimated to be about 6 hours. Styrene is not thought to bioaccumulate or bioconcentrate in organisms and food-chains to any measurable extent *(18)*.

Food

Styrene has been detected as a natural constituent of a variety of foods and beverages, the highest levels occurring in cinnamon *(13)*.

Polystyrene and its copolymers are widely used as food-packaging materials. The ability of styrene monomer to migrate from polystyrene packaging to food has been reported in a number of publications and probably accounts for the greatest contamination of foods by styrene monomer. In dairy products, styrene has been reported to convey disagreeable odours and taste at 0.2–0.5 mg/kg *(18)*. Styrene has been found at concentrations of 2.5–80 µg/kg in yoghurt and other milk products packed in polystyrene containers, the styrene content in the products increasing in the course of storage *(18)*.

The United States Food and Drug Administration in 1993 established regulations for the use of polymers and copolymers of styrene in products in contact with food. 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% *(13)*.

Estimation of various sources to daily intake

Occupational exposures to styrene far exceed any other sources of exposure. In the general population, indoor and outdoor air account for the largest exposures. However, smokers inhale relatively high amounts of styrene in both mainstream and sidestream cigarette smoke. Daily styrene intake for cigarette smokers consuming 20 cigarettes/day has been estimated at 2.81 µg/kg body weight for adults of 20–70 years and 3.51 µg/kg body weight for adolescents of 12–19 years *(11)*. The daily personal intake of styrene from dietary sources has been estimated to be 1–4 µg in the United Kingdom *(24)* and 9 µg in the United States *(25)*. Daily intake of styrene was estimated in Canada for different age groups *(11)*. Besides the level absorbed via smoking, the daily total styrene dose of an adult was assumed to be less than 0.22–0.33 µg/kg body weight (bw); food (0.11 µg/kg bw) and ambient air (0.005–0.13 µg/kg bw; 0.08 µg/kg for indoor air) were considered to be the main sources. To illustrate the relative significance of various sources of exposure to styrene, WHO *(18)* estimated exposure levels in several environments and compared nominal daily intakes from those sources; the results are summarized in Table 2.

Table 2. Estimated daily 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 litres/day)	1 µg/litre	2 µg
Cigarette smoke (20 cigarettes/day)	20–48 µg/cigarette	400–960 µg

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

Source: WHO Regional Office for Europe *(18)*; see also Fishbein *(26)*.

Toxicokinetics

The pharmacokinetics and metabolism of styrene have been reviewed *(13)*.

Absorption

In animal and human controlled studies, the uptake of styrene has been found to be rapid. The principal routes of exposure are pulmonary and, to a lesser extent, dermal. In several studies with workers and volunteers, the pulmonary retention of styrene was 60–70% of the inhaled dose.

Styrene in ambient air is absorbed through the skin at 2–5 % of the dose absorbed in the respiratory tract. Liquid styrene was found to penetrate the skin at a rate of 1 µg/m^2 per minute *(13)*.

Distribution

Styrene is widely distributed throughout the body. The distribution and sequestration of styrene to fat tissue and its subsequent slow elimination indicate a potential for accumulation in situations of repeated daily exposure. However, in a study of workers exposed to 37 ppm (160 mg/m^3; 8-hour TWA) styrene, no evidence of accumulation was found in monitoring samples during a working week *(27)*.

Biotransformation

The metabolic pathways of styrene have been reviewed *(13)*. Styrene is oxidized to styrene-7,8-oxide by the cytochrome P-450-mediated monooxygenase system. The responsible isozymes are the ethanol-inducible CYP2E1, CYP2B6 and CYP1A2, and additional isozymes with lower activity for styrene may be involved *(28)*. Styrene can also undergo oxidation also by other mechanisms and it can be cooxidized to styrene-7,8-oxide during the lipoxynase-mediated formation of arachidonic acid peroxides *(29)*. *In vitro*, styrene oxidation to styrene-7,8-oxide has been shown to be mediated by oxyhaemoglobin and myoglobin *(30,31)*.

Enzymatic hydrolysis of styrene-7,8-oxide yields phenylethylene glycol which is further oxidized to mandelic acid and phenylglyoxylic acid, the principal urinary metabolites of styrene in humans *(13)*. Conjugation of styrene-7,8-oxide with glutathione leads to urinary excretion of thioethers or mercapturic acid, quantitatively a minor metabolite in humans. In a field study carried out on workers exposed to 10–73 ppm of styrene, styrene-7,8-oxide concentration in blood was found to be linearly correlated with ambient styrene *(32)*.

Elimination

The elimination of styrene is linear at lower concentrations of atmospheric styrene. At concentrations of less than 200 ppm there is a fast elimination phase with a half-life of 0.5–0.7 hours, and a second phase of slow elimination with a half-life of 13 hours *(13)*. Several physiologically-based pharmacokinetic models have been developed *(33,34)* which simulate the behaviour of styrene and styrene-7,8-oxide in the body. Partially saturated metabolism can be predicted by the models at high exposures exceeding 300 ppm (1600 mg/m^3).

Urinary excretion of mandelic acid and phenylglyoxylic acid is biphasic. After an 8-hour exposure of workers to styrene at concentrations of 26–130 mg/m^3 (6.1–30.5 ppm), both metabolites have a half-life of about 2.5 hour for the first phase and 30 hours for the second *(35)*. These compounds are used in assessing occupational exposure to styrene. According to several studies, an exposure to 20 ppm (80 mg/m^3) styrene corresponds, the following morning, to a combined mandelic acid and phenylglyoxylic acid level of about 2.9 mmol/litre *(27)*. Coexposure to ethanol inhibits the metabolism of styrene, resulting in a lag in the excretion of mandelic acid *(36)*.

Health effects

Effects on experimental animals and *in vitro* test systems

Toxicological effects

Acute exposure of animals to styrene vapour causes irritation of the skin, eyes and respiratory tract and central nervous system (CNS) effects. Liquid styrene is a skin irritant which, on direct contact, causes erythema. Single exposures of rats and guinea-pigs to styrene at a concentration of 1300 ppm (5633 mg/m^3) resulted in CNS effects, including weakness and unsteadiness. Following exposure to 2500 ppm (10.8 g/m^3) styrene for 10 hours, rats and guinea-pigs lost consciousness; exposure to 5000-10 000 ppm (21.7–43.3 g/m^3) resulted in unconsciousness and death. The principal pathological findings in these animals were severe pulmonary irritation, congestion, oedema, haemorrhage and leukocyte infiltration *(13,37)*. Early studies have reported LC_{50} values ranging from 2700 ppm (11 500 mg/m^3; 4-hour exposure) to 4618 ppm (19 650 mg/m^3; 6-hour exposure) for rats and from 2429 ppm (10 340 mg/m^3; 6-hour exposure) to 4930 ppm (21 000 mg/m^3; 2-hour exposure) for mice *(38)*. Recent findings have suggested that styrene is more toxic to mice than to rats. A 6-hour exposure to inhaled styrene at 500 ppm (2130 mg/m^3) or 2 × 250 ppm (1065 mg/m^3) resulted in the deaths of 30% and 44%, respectively, of male B6C3F$_1$ mice *(39)*; the dead mice showed centrilobular coagulative necrosis of the liver. The oral LD_{50} of styrene was reported to be about 5000 mg/kg body weight in rats and 320 mg/kg body weight in mice *(38)*.

Long-term contact with styrene results in blistering of the skin and development of dermatitis, which is thought to be due to defatting of the skin *(13)*. Exposure of B6C3F$_1$ mice to 250 ppm (1065 mg/m^3) or 500 ppm (2130 mg/m^3) of styrene for 6 hours/day for 5 or 14 days induced

hepatic necrosis; the parental strain C57BL/6 was equally sensitive, but strain DBA/2 was clearly less susceptible to styrene-induced hepatotoxicity *(39,40)*. Morphological changes were observed in the rat kidney *(41)* and in the respiratory mucosa of rats *(42)* and mice *(43)* after inhalation exposure to styrene. Epithelial changes, including vacuolization, nuclear pycnosis, and exfoliation of epithelial cells, were seen in the nose and trachea of rats exposed by inhalation to styrene at a concentration of 800 ppm (3466 mg/m^3) for hours/day for 8 weeks *(44)*. Effects in the liver, kidney and lungs of rats were associated with depletion of glutathione *(13,45,46)*; the direct toxicity of glutathione conjugates may play a role in the kidney *(47)*.

Reproductive and developmental toxicity

Styrene has been shown to cross the placental barrier both in rats and in mice but standard teratological studies carried out with styrene revealed negative results in rats and rabbits. These studies have been reviewed in detail *(13)*. Damage to seminiferous tubules and decreased sperm counts have been observed in male rats exposed to styrene by gavage at a rate of 400 mg/kg body weight daily for 60 days *(48)*; in young rats exposed similarly during the first 60 days of their life, the effects were observed at a daily dose of 200 mg/kg *(49)*. In *in vitro* cultures of postimplantation rat embryos, styrene was embryotoxic at lower concentration (1 mmol/litre) than benzene, toluene or xylene *(50)*.

Kishi et al. *(51)* reported that *in utero* exposure (days 7–21 of gestation) of rats to 60 and 300 ppm (260 and 1280 mg/m^3) reduced pup body weight and (at 300 ppm) induced alterations in behaviour and neurotransmitter levels in the brain at concentrations that did not affect the dams. Behavioural tests on the pups indicated that 60 ppm (260 mg/m^3) delayed the development of the righting and auditory startle reflexes. Postnatal exposure (for 48 days from birth) of rats to 50 ppm for 7 hours/day resulted in behavioural changes *(52)*. Beliles et al. *(53)* observed that pup survival or weight were reduced in each of three generations of rats exposed to styrene in drinking-water (250 mg/litre) over the life span.

Neurotoxic effects

Early studies on the neurotoxicity of styrene gave equivocal results *(7)*. The limited data available on animal experiments indicate that neurological development is among the most sensitive endpoints to the effects of styrene exposure; these studies are discussed in the previous section (Reproductive and developmental toxicity).

Mild neurobehavioural disturbances were seen in rats exposed to 1400 ppm (6000 mg/m^3) for 18 weeks *(54)* and in mice exposed to 425 ppm (1841 mg/m^3) for two weeks *(55)*.

A consistent dose-dependent decrease of dopamine and disturbance of dopaminergic brain functions were seen in rabbits exposed to 1500 ppm for 12 hours/day for 7 days *(56)*. The levels of norepinephrine were unchanged, suggesting that the styrene metabolites phenylglyoxylic acid and mandelic acid condense with dopamine and deplete it *(57)*. In protein-deficient young rats exposed to styrene, decreases in dopamine, norepinephrine and serotonin were observed in association with increased aggressive behaviour and amphetamine-induced locomotor activity *(58)*.

Styrene produced ototoxicity in 5 days when administered to rats by inhalation for 8 hours/day at 1000 ppm *(59)* and at lower concentrations when administered for a longer period of time *(60,61)*. Inhalation exposure of adult male rats to 1600 ppm of styrene for 5

days impaired the auditory functions *(62)*, and in a combined exposure of rats to styrene and industrial noise induced a severe flat hearing loss *(63)* while styrene alone was ototoxic without apparent hearing loss.
Styrene was cytotoxic to murine neuronal cells in culture at concentrations in excess of 2 mmol/litre, but no changes in action potential production were observed *(64)*.

Macromolecular adducts and genotoxic effects
Styrene-7,8-oxide, the metabolite of styrene, reacts with DNA mainly at the *N*-7 position in guanine, but also at other sites and with other bases. Substitution occurs at both the α- and β-positions of the styrene molecule. Experiments with radiolabelled styrene and styrene-7,8-oxide have shown that both have a low level of DNA binding activity in experimental animals, styrene probably after biotransformation into styrene-7,8-oxide. ^{32}P-postlabelling studies have demonstrated the potential of the technique to detect styrene-DNA adducts *(65,66)*.

Styrene-7,8-oxide alkylates several nucleophilic sites in proteins, particularly cysteine sulfhydryl, histidine imidazole, lysine amino, aspartic and glutamic carboxylic groups, and the *N*-terminal position. In experimental animals, styrene oxide treatment results in cysteine adducts in haemoglobin and albumin, and valine and carboxylic acid adducts in haemoglobin. The available evidence indicates that the extent of alkylation is low and thus styrene and styrene-7,8-oxide have low DNA and protein binding activities *in vivo (65)*. In a comparative study in mice and rats on adducts of *N*-terminal valine of haemoglobin, the adduct levels were two to three times higher in the mouse than in the rat *(67)*.

The genotoxic effects of styrene have been reviewed recently *(13,68,69)*. Styrene is genotoxic following metabolic activation. Its reactive metabolite is styrene-7,8-oxide, a direct-acting genotoxin.

Styrene is genotoxic *in vitro* in assays that allow effective metabolic activation *(13,68,69)*. Gene conversion and mitotic recombination were induced by styrene in logarithmically growing yeast, with inherent metabolic activation capacity; gene conversions in yeast were also increased in a host-mediated assay with the mouse as the host. Styrene elevated the frequency of chromosome aberrations and sister chromatid exchanges (SCEs) in human lymphocyte cultures *in vitro* in the presence of erythrocytes, probably owing to metabolic activation mediated by oxyhaemoglobin *(70–72)*. SCEs were also produced by styrene in whole-blood lymphocyte cultures of rat, and in Chinese hamster ovary CHO cells when cyclohexene oxide was used to inhibit epoxide hydrolase activity in S9 mix or when human erythrocytes were used as a metabolizing system. 6-Thioguanine-resistant mutants were induced in Chinese hamster V79 cells in a rat liver perfusion system containing rat erythrocytes.

Styrene was not genotoxic in various studies with bacteria in the presence or absence of fortified postmitochondrial fraction from rodent liver (S9 mix), with a few exceptions *(13,69)*. Other tests for genotoxicity *in vitro*, in yeasts and mammalian cells, using S9 mix for metabolic activation, also gave negative results, excluding a few weak responses *(13,69)*.

Weak but consistent induction of SCEs by styrene in various tissues was observed in several studies in mice and rats after intraperitoneal (i.p) or inhalation exposure *(13)*, with lowest effective doses (LED) ranging from 450 mg/kg in mouse splenocytes to 750 mg/kg in rat

splenocytes *(73)* or from 225 ppm for 2 weeks in rat lymphocytes *(74)* to 850 ppm for 4 days in mouse bone marrow and liver *(75)*. Styrene-induced DNA single-strand breakage in various organs of mice, LED 170 mg/kg i.p. *(76)* but not in rat lymphocytes at up to 488 ppm for 2 weeks *(74)*. Styrene, administered i.p. or by inhalation did not induce chromosome aberrations in rodent bone marrow, splenocytes or lung cells, except in one study in rat bone marrow following inhalation exposure to 300 ppm for 9–11 weeks *(13,77)*. In mice, micronuclei were weakly increased in bone marrow erythrocytes in two studies where styrene was administered i.p., LED 250–600 mg/kg *(73,78)*, but not in another study where micronuclei were examined in erythrocytes or splenocytes following a 2-week inhalation exposure at concentrations of up to 500 ppm *(79)*. Two reports on micronucleus induction in rat bone marrow erythrocytes with up to 3000 mg/kg i.p. *(73)* or lymphocytes at 488 ppm for 2 weeks *(74),* and one in Chinese hamster bone marrow erythrocytes with 1000 mg/kg *(80)* showed no genotoxic activity for styrene.

In summary, genotoxicity assays on styrene in experimental animals have given conflicting results. The findings suggest a weak genotoxic activity for styrene *in vivo*, as revealed by the most sensitive techniques (SCEs and DNA strand breakage), especially in the mouse – the rodent species expected to be more susceptible than the rat or Chinese hamster to styrene.

Carcinogenic effects in animals

The carcinogenicity of styrene in animals has been reviewed by IARC *(13)*. Jersey et al. *(81)* exposed male and female Sprague-Dawley rats to styrene at a concentration of 600 or 1200 ppm (2556 or 5112 mg/m^3) styrene for 6 hours/day for 5 days/week, until 50% survival (males, 18.3 months; females, 20.7 months). The higher exposure was reduced to 1000 ppm after 2 months because of narcosis. There was some evidence of carcinogenicity in the females. In the two exposed groups, 6 of the 85 females had leukaemia-lymphosarcomas versus one of 85 controls; the result was statistically significant only in comparison with historical controls. The results in males were flawed because of chronic murine pneumonia which caused a high rate of mortality in both control and exposed male rats.

In another study on Sprague-Dawley rats, inhalation exposures to styrene at concentrations of 25, 50, 100, 200 or 300 ppm (107, 213, 426, 852 or 1278 mg/m^3) for 4 hours/day for 5 days/week for 52 weeks were studied *(82,83)*. The combined incidence of benign and malignant mammary tumours was greater in treated female rats than in controls: 34/60 controls, 24/30 at 25 ppm, 21/30 at 50 ppm, 23/30 at 100 ppm, 24/30 at 200 ppm and 25/30 at 300 ppm ($P = 0.01$, Fisher exact test, highest dose compared to controls). The incidence of malignant mammary tumours was significantly increased in treated females: 6/60 controls, 6/30 at 25 ppm, 4/30 at 50 ppm, 9/30 at 100 ppm, 12/30 at 200 ppm and 9/30 at 300 ppm ($P < 0.01$, Cochran-Armitage trend test). The high background incidence of mammary tumours makes the interpretation of these results difficult. The study also included exposure of male and female Sprague-Dawley rats to daily styrene doses (in olive oil) of 50 and 250 mg/kg bw, for 4–5 days/week for 52 weeks; no treatment-related increases in tumour incidence were reported *(83)*.

Ponomarkov and Tomatis *(84)* administered styrene to pregnant O20 (1350 mg/kg body weight) and C57BL (300 mg/kg) mice, and BDIV rats (1350 mg/kg) by a single gastric administration in olive oil on day 17 of gestation. The progeny of these dams received styrene (strain O20, 1350 mg/kg; strain C57BL, 300 mg/kg; strain BDIV, 500 mg/kg) by gastric intubation once a week from weaning until 16 (O20) or 120 (C57BL and BDIV) weeks of

age. In the experiment with strain O20, the treatment was stopped at week 16 because of toxicity; the animals were examined at week 120. The authors found weak evidence of carcinogenicity in the O20 mice, based on an increased incidence of lung tumours: the combined incidence of lung adenomas and carcinomas was significantly ($P < 0.01$) increased over that in vehicle controls: males 8/19 versus 20/23, and females 14/21 versus 32/32. The C57BL mice and BDIV rats showed no treatment-related increases in tumour incidence.

The US National Cancer Institute (NCI) conducted an oral gavage study in male and female rats and mice on a mixture of styrene (70%) and β-nitrostyrene (30%) in corn oil *(85)*. The doses were 87.5 and 175 mg/kg per day in mice and 150 and 300 mg/kg per day in rats. Both species were exposed for 3 days/week for 78–79 weeks, followed by an additional observation period of 14 weeks (mice) or 29 weeks (rats). The combined incidences of adenoma and carcinoma of the lung in males were 0/20 in the controls, 11/44 in the low-dose group ($P = 0.016$, Fisher exact test), and 2/43 in the high-dose group. No other significant treatment-related increases in tumours were observed. The study was flawed because of the small numbers of controls (20), an inadequate exposure period (18 months), and exposure to a mixture, not to styrene alone.

In another NCI study *(86)* daily oral styrene doses (in corn oil) of 150 or 300 mg/kg were administered to $B6C3F_1$ mice and of 500, 1000, or 2000 mg/kg to rats for 5 days/week for 78 weeks (for 103 weeks in rats receiving 500 mg/kg). The mice were held unexposed for an additional 13 weeks, and mid- and high-dose rats for 27 weeks. A significant ($P = 0.02$; Cochran-Armitage test) increase was observed in the incidence of lung tumours in male mice (0/20 controls, 6/44 low dose, 9/43 high dose). The mean incidence of such tumours in historical controls was 12% (20% in two control groups). A significant ($P = 0.034$; Cochran-Armitage test) increasing trend in the incidence of hepatocellular adenoma was also observed in female mice (0/20 controls, 1/44 low dose, 5/43 high dose). No treatment-related increases in the incidence of tumours were observed in the rats.

Beliles et al. *(53)* reported a study in male and female Sprague-Dawley rats in which styrene was continuously administered in drinking-water (125 and 250 mg/litre) for 104 weeks. No evidence of carcinogenicity was seen. As the exposure levels were well below the maximum tolerated dose, the study is not adequate for evaluating the carcinogenicity of styrene.

In conclusion, there is little evidence for a carcinogenic action of styrene in animals. The available studies all have deficiencies in design, conduct and reporting.

Effects in humans

Irritation and toxic effects

Styrene has been described to cause subjective symptoms of irritation of the eyes, throat and respiratory tract at approximate concentrations of 10–100 ppm (43–426 mg/m^3) or higher *(7,13,38,87)*. Subjective health complaints were usually not seen in the glass-reinforced plastics industry with concentrations of styrene below 24 ppm (102 mg/m^3) *(13,88)*.

Exposure to 100 ppm (426 mg/m^3) and above caused acute irritation of mucous membranes in the eyes and the upper respiratory tract; in reinforced plastics workers these levels of styrene were associated with chronic bronchitis and obstructive pulmonary changes. Cases of styrene-induced asthma and contact dermatitis have also been reported *(13)*.

Styrene-exposed reinforced plastics workers have been described to show altered distribution of peripheral blood lymphocyte subsets. In workers exposed to styrene concentrations of 10–50 ppm (43-213 mg/m^3), T helper-inducer lymphocytes were reduced in number, while natural killer cells were increased *(89)*.

Sensory effects

The odour threshold for styrene is only 0.016 ppm (70 μg/m^3). Its characteristic pungent odour is recognized at concentrations 3–4 times higher than this threshold value. Some individuals can perceive the odour at levels lower than 70 μg/m^3, but, in general, odour problems are not likely to occur if peak concentrations in the ambient air are kept below this threshold value. When styrene is emitted into the air, its half-time is estimated to be 2 hours. In ambient air it is chemically transformed into benzaldehyde and formaldehyde, both of which are odorous air pollutants *(18)*.

Neurotoxic effects

The neurotoxicity of styrene has recently been reviewed *(90)*. Styrene, like many other lipid-soluble organic solvents, can be acutely neurotoxic at high concentrations. Such effects at high concentrations do not necessarily imply that styrene would also produce reversible or irreversible damage to the nervous system at lower exposure levels. Over the last 20 years, a number of epidemiological studies have suggested that styrene is associated with neuropsychological deficits, such as slowing of reaction time and vestibulomotor dysfunction, at exposure levels of around or more than 50 ppm (210 mg/m^3). Somewhat lower concentrations appear to be associated with such effects as loss of colour vision. The effects that have been registered, are "subclinical", but measurable.

The impairment of colour vision among reinforced plastics workers has been shown in different populations at exposure levels starting from 25–50 ppm (107–213 mg/m^3) *(91–95)*. Some studies have suggested reduced peripheral nerve conduction velocity, especially in sensory nerves, at styrene exposure levels of 50–100 ppm (213–426 mg/m^3) *(96–98)*.

Several studies among workers employed in the production of glass-fibre reinforced by polyester resin revealed that styrene can cause depression of the CNS. Prenarcotic symptoms such as weakness, headache, fatigue, malaise, tension, nausea and dizziness were reported where styrene concentrations in the air exceeded 50 ppm (213 mg/m^3) *(7)*. As exposure increased to more than 200 ppm (852 mg/m^3) drowsiness, nausea and disturbances in equilibrium become evident.

Slight disturbances of visuomotor accuracy and psychomotor performance have been noted at styrene levels exceeding 50 ppm *(99,100)*. Verbal learning skills were impaired in reinforced plastics workers with a sum of the urinary styrene metabolites (mandelic acid and phenyl glyoxylic acid) of more than 150 mmol/mol of creatinine (said to correspond to an airborne styrene level of 25 ppm, 107 mg/m^3), while logical memory and visuoconstructive abilities were reported to be affected in workers with a urinary metabolite sum of 300 mmol/mol of creatinine (corresponding to 50 ppm, 213 mg/m^3, of styrene in air) *(101)*. Slowing of single reaction times has been observed in styrene-exposed workers exposed to average concentrations in the range 50–100 ppm (213–426 mg/m^3) *(97,102–105)*. Increased incidence of abnormalities in electroencephalograms has been reported in reinforced plastics workers at styrene exposure levels of less than 100 ppm *(106,107)*.

Female reinforced plastics workers with average exposure to styrene at concentrations of about 130 ppm (554 mg/m^3) showed an increase in serum levels of prolactin and growth hormone *(108)*, and women occupationally exposed to styrene also had an exaggerated prolactin response to administration of thyrotropin-releasing hormone *(109)*. The levels of serum prolactin and thyroid-stimulating hormone and the prolactin response correlated positively with urinary excretion of the styrene metabolites mandelic acid and phenyl glyoxylic acid. The findings were considered to be consistent with tubero-infundibular dopamine depletion, which has been observed in rabbits exposed to styrene. The level of platelet monoamine oxidase, an enzyme involved in dopamine catabolism, has been reported to be inversely related to styrene exposure in reinforced plastics workers *(110,111)*.

In summary, prenarcotic symptoms and altered coordination have commonly been reported in workers and volunteers exposed to styrene at concentrations of 10–100 ppm (43–426 mg/m^3) or more. Some studies have indicated increased prevalence of abnormal EEG patterns, reduced peripheral nerve conduction velocity, and neuroendocrine effects in workers exposed to air styrene concentrations of 50–100 ppm (213–426 mg/m^3). In neuropsychological studies, the principal finding has been a slowing of reaction time at occupational exposure to 50–100 ppm (213–426 mg/m^3). More subtle effects, such as reductions in visuomotor accuracy and verbal learning skills, and subclinical effects on colour vision, appear to occur at lower concentrations (25–50 ppm, 107–213 mg/m^3*)*.

Macromolecular adducts and genetic toxicity

The genetic toxicology of human styrene exposure and styrene macromolecular binding have recently been reviewed *(13,65,68,69)*. The genotoxic effects of styrene in humans have mostly been studied in the reinforced plastics industry where styrene is the main chemical exposure. Only a few studies are available on other branches of industry, such as styrene production, where exposure to styrene is low. Exposure levels in the studies, evaluated on the basis of urinary styrene metabolites or workplace air samples, have ranged widely as can be seen from Tables 3 and 4.

The levels of the *N*-terminal valine adduct of haemoglobin, *N*-(1-hydroxy-2-phenyl-ethyl)valine, have been found to be four times higher in styrene-exposed workers than in controls, In a Swedish-Finnish study *(112)*, increased levels of adducts to *N*-terminal valine in haemoglobin were seen in reinforced plastics workers (mean 28 pmol × g^{-1} globin) in relation to control persons (mean $\leq$13 pmol × g^{-1} globin). This level was low, while a background adduct level was also seen among the controls. A significant correlation of individual adduct levels and free styrene-7,8-oxide and styrene glycol in blood, as well as mandelic acid in urine, was seen in a regression analysis. Extrapolation from the metabolite monitoring data gave an estimate of about 75 ppm (320 mg/m^3) for the workplace air styrene concentration. In another study, using similar techniques, but at lower exposure levels (7 ppm, 30 mg/m^3), no styrene-7,8-oxide adducts in *N*-terminal valine of haemoglobin were seen *(113)*.

The first DNA adducts studies on humans exposed to styrene were published by Liu et al. *(114)* who detected styrene-7,8-oxide modified guanine adducts in a single exposed person but none in a single unexposed individual. Extensive studies on biomonitoring of styrene exposure using DNA adducts have been performed *(11,116)*. Lamination workers from work sites representing high exposures (mean air styrene level 87 ppm, 370 mg/m^3) and moderate exposures (mean air styrene level 49 ppm, 210 mg/m^3) were studied.

Table 3. Results of biomonitoring studies on DNA damage and mutations in relation to exposure to styrene

Endpoint		Length of exposure (years)		Styrene in air (ppm)		Urinary mandelic acid (mg/g creatinine)		Result	Reference
No. exposed	No. of referents	Range	Mean	Range	Mean	Range	Mean		
DNA adducts in leukocytes									
23	8	n.d.	6–2	<50–168	50–89	n.d.	336–386	+	*(115)*
47[a]	n.d.	n.d.	n.d.	0.3–55	15	n.d.	n.d.	+	*(118)*
9	9	n.d.	6.7	10–54	29	60–342	160	+	*(117)*
9	8 (7)[b]	15–17	5.2	8–59	25	52–1332	227	+	*(119)*
7[b]	8	1–22	8.3	<1	<1	n.d.	n.d.	+	*(119)*
DNA strand breakage in leukocytes									
17	17	n.d.	6.7	n.d.	n.d.	152–3271 mg/l	1430	+	*(120)*
14	8	n.d.	2.7	1–44	11.2	96–2496	243	?	*(121)*
17[c]	n.d.	0–25	n.d.	0.04–20	7	0–261	70	+	*(122)*
9	7	1.5–17	9	8–59	25	52–1332	227	+	*(119)*
Unscheduled DNA synthesis in leukocytes									
38	20	1–23	8.1	1–40	13	n.d.	n.d.	+[d]	*(123)*
38	20	1–23	8.1	1–40	13	n.d.	n.d.	–[e]	
11	9	n.d.	2.7	1–44	11.2	96–2496	243	+[c]	*(121)*
Glycophorin A mutant erythrocytes									
15	15[f]	>1	n.d.	n.d.	32	n.d.	n.d.	(+)[g]	*(124)*
9	13[f]	>1	n.d.	n.d.	n.d.	n.d.	n.d.	(+)[h]	
47	47	n.d.	8.5	6–112	36	61–2570 mg/l	669 mg/l	+	*(125)*
HPRT mutant lymphocytes									
45[i]	1	4–31	20	0–140	20	n.d.	n.d.	?	*(126)*
9	8 (7)[b]	1.5–17	9	8–59	25	52–1332	227	(+)	*(119*

+, positive result; (+) weakly positive result; –, negative result; ?, inconclusive result; n.d., no data

[a] Evaluation was based on regression between exposure measurements and adduct levels and no controls were used.

[b] Factory controls.

[c] The same persons served as their own controls before the shift.

[d] *N*-acetylaminofluorene-induced unscheduled DNA synthesis.
[e] UV-induced unscheduled DNA synthesis.
[f] Consisted of persons who had never worked with styrene or had had styrene exposure in the past.
[g] Using old-type flow cytometer; positive results for Nφ variants and negative results for N/N variants, but authors indicated that the groups compared were not well matched for age and smoking.
[h] Using new-type flow cytometer; positive results for Nφ variants and negative results for N/N variants, but authors indicated that the groups compared were not well matched for age and smoking.
[i] Coexposure to dichloromethane.

Table 4. Results of cytogenetic biomonitoring studies in relation to exposure to styrene

No. exposed	No. of referents	Length of exposure (years)		Styrene in air (ppm)		Urinary mandelic acid (mg/g creatinine)		Result of cytogenetic studies			Reference
		Range	Mean	Range	Mean	Range	Mean	CA	MN	SCE	
10	5	0.6–8.5	3.2	Up to 300	n.d.	23–3257	721	+	+	n.d.	*(127)*
16	6	1–15	6.3	Up to 300	n.d.	23–3257	570	+	n.d.	n.d.	*(128)*
10	6	2–16	7.8	Up to 300	n.d.	23–3257	609	n.d.	n.d.	–	
11	3	1–15	6.2	Up to 300	n.d.	52–1646	350	+	n.d.	n.d.	
5	20	14–25	21.6	0–6.8	0.5	19–40 mg/l	30	–	n.d.	n.d.	*(129)*
12	20	3–39	20.3	0–47	1.8	<5–100 mg/l	32	–	n.d.	n.d.	
14	20	2–24	7.9	<50–300	113	42–>1500	593	+	n.d.	n.d.	
12	12	3–34	20.3	0–9	n.d.	10–109 mg/l	n.d.	–	n.d.	n.d.	*(130)*
6	6	0.5–10	4	14–192	39	225–2100	490	+	n.d.	n.d.	*(131)*
24	24	4–27	14.4	0.7–178	58.1	0–320	n.d.	(+)[a]	n.d.	n.d.	*(132)*
36	37	0.3–12	5	0–237	47	n.d.	n.d.	+	n.d.	n.d.	*(133)*
20	21	0.3–12	5	0–237	47	n.d.	n.d.	n.d.	n.d.	(+)	
16	13	0.6–9.3	4.5	1–211		90–4300 mg/l	594	–	n.d.	–	*(134)*
18	6	0.2–30	8.6	40–50	n.d.	0–1041 mg/l	332	(+)[b]	n.d.	–	*(135)*
38	20	1–23	7.9	1–36	13	9–316	65	n.d	+	n.d.	*(136)*
24 (22)[c]	21 (20)[c]	1–22	9.4	7–>96	n.d.	45–1108 mg/l	458 (a.m.)	+	n.d.	+	*(137)*
42 (37)[c]	30 (28)[c]	1–22	n.d.	7–>96	n.d.	45–1440 mg/l	479 (a.m.)	+	n.d.	+	*(138)*
18	9	n.d.	n.d.	2–44	13.2	n.d.	n.d.	+	n.d.	–	*(139)*
15	13	1–26	11.2	n.d.	24	<152–304	n.d.	–	n.d.	n.d.	*(140)*
12	12	1–26	n.d.	n.d.	24	<152–304	n.d.	n.d.	+	n.d.	
36	19	1–11	n.d.	1–236	n.d.	35–972 μg/l	n.d.	?[d]	n.d.	n.d.	*(141)*
22	22	1–11	n.d.	9–132	n.d.	40–3000 μg/l	n.d.	?[d]	n.d.	n.d.	
21	21	1–25	6.7	8–63	24	0–1103	243	–	–	–	*(142)*
32	32	n.d.	18.8	0.4–58	n.d.	n.d.	n.d.	+	n.d.	n.d.	*(143)*
8	8	n.d.	4.5	10–46	n.d.	n.d.	n.d.	+	n.d.	n.d.	
11	11	n.d.	10	28–140	61	n.d.	n.d.	–	n.d.	n.d.	*(144)*
74 (20)[e]	20 (20)[e]	n.d.	n.d.	<0.02–1.4	n.d.	n.d.	n.d.	–	n.d.	–	*(145)*

11	14	0.1–25.4	8.1	1–39	13	<6–317	128	–	n.d.	n.d.	*(146)*
20	22	0.1–25.4	8.1	1–39	13	<6–317	128	n.d.	–	n.d.	
7	8	n.d.	8.6	1.7–131	50	n.d.	275	n.d.	n.d.	–	*(147)*
13	12	n.d.	7.2	5.8–130	55	n.d.	323	n.d.	n.d.	–	
48	0	n.d.	n.d.	0.2–55.3	15.1	n.d.	n.d.	n.d.	n.d.	+[f]	*(148,149)*
11	11 (smokers)	n.d.	6.4	n.d.	n.d.	<152–3271	1674	–	–	–	*(120)*
6	6 (non-	n.d.	7.2	n.d.	n.d.	<152–2526	989	+	–	–	
17	smokers)	n.d.	6.7	n.d.	70	<152–3271	1430	–	–	–	
	17 (all)										
10	9	n.d.	2.7	1–44	17.2	96–2496	243	n.d.	+	–	*(121)* *(150)*
50	54	n.d.	n.d.	5–182	43	n.d.	n.d.	–	–	–	*(22)*
25	54	n.d.	n.d.	1–133	11	n.d.	n.d.	–	–	–	
7	7	1–18	n.d.	5–24	n.d.	46–345	186	–	–	n.d.	*(151)*
11	11	1.5–15	n.d.	27–104	n.d.	423–1325	725	+	–	n.d.	
14	20	1–26	8.0	31–42	40	100–1610 mg/l	652	n.d.	n.d.	+	*(152)*
9	20	2–27	8.8	13–21	20	100–400 mg/l	187	n.d.	n.d.	+	
14	20	n.d.	n.d.	n.d.	10	n.d.	n.d.	n.d.	n.d.	–	
52	24	n.d.	n.d.	0.5–26	7.3	11–649	102	n.d.	–	–	*(153)*
46[g]	23	4–31	20	0–140	20.7	n.d.	n.d.	+	+	+	*(126)*
23 (22)[c]	51 (34)[c]	n.d.	n.d.	0.5–28	n.d.	n.d.	n.d.	+	n.d.	+	*(154)*
23 (22)[c]	51 (34)[c]	n.d.	n.d.	20–326	n.d.	n.d.	n.d.	+	n.d.	+	
18	18	10–22	14.3	n.d.	n.d.	145–1204	328	+	–	n.d.	*(155)*

CA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchange; +, positive result; (+) weakly positive result; –, negative result; ?, inconclusive result; n.d., no data.

[a] Positive for gaps (see *156*).

[b] Borderline positive for gaps.

[c] For SCE analysis.

[d] Increase reported in gaps, but conclusion negative.

[e] Styrene production; coexposure to low levels of benzene; values in parenthesis show numbers of persons in another sampling.

[f] Based on regression analysis.

[g] Coexposure to dicloromethane.

Using synthetic standards and the ^{32}P-postlabelling technique, O^6-(2-hydroxy-1-phenylethyl)-2'-deoxy-guanosine-3'-monophosphate (O^6-dGMP) adducts were detected among the exposed workers, at 7–16 times higher level than in the controls.

In a further study, where DNA adducts were investigated before and after a 2-week vacation, no decline in adduct levels was seen, indicating a slow removal of the specific O^6-guanine adducts of styrene from DNA *(117)*. Horvath et al. *(118)* studied DNA adducts by postlabelling in mononuclear blood cells of lamination workers and found two types of adducts, one identified as guanine N^2 adduct, to be increased with a significant linear relationship to individual measurements of airborne styrene, but not to SCE frequencies among the same workers. DNA adduct levels were highly elevated among the styrene exposed workers, with a correlation to DNA strand breakage measured by the comet assay, but not to mutant frequencies (hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus) *(119)*. These results suggest that no simple quantitative relationships exist between the adducts and other parameters of DNA damage in human lymphocytes. In this study, control subjects from the administration of the same plastics factory also showed elevated adduct levels in comparison with unexposed control persons. The DNA adduct studies are summarized in Table 3 *(115, 117–126)*.

Studies on cytogenetic parameters – chromosomal aberrations, SCEs, and micronuclei – in peripheral lymphocytes of workers employed in the reinforced plastics industry are summarized in Table 4 *(22,120,121,127–156)*. Positive findings have primarily concerned chromosomal aberrations, which have been observed to be increased in the majority of the studies. SCEs and micronuclei were also found to be increased in some studies.

On the basis of rough exposure estimates and observed frequencies of chromosomal aberrations, the “lowest observable effect level" for long-term styrene exposure has been proposed to be 20–30 ppm (85–128 mg/m^3) *(157)* or 50 ppm (213 mg/m^3) *(68)*. A recent meta-analysis on cytogenetic data from 25 reports on occupational styrene exposure *(158)* showed a significant increase in weighed frequency ratio for chromosomal aberrations from studies with median styrene exposure above the chosen dichotomization point, 125 mg/m^3 (30 ppm); results for SCEs and micronuclei were inconclusive. Fleig and Thiess *(129)* observed an increase in chromosomal aberrations in a worker group with high exposure to styrene (mean 114 ppm, 486 mg/m^3) but not in two other groups with lower exposure levels (means 0.5 and 1.8 ppm, 2 and 8 mg/m^3). Anderson et al. *(133)* observed an increase in the frequency of chromosomal aberrations with increasing total styrene exposure (expressed as the average concentration in mg/m^3 multiplied by the number of years of employment) in a low-dose group (mean total styrene exposure 137 mg/m^3) but not in a high-dose group (mean 1204 mg/m^3). Forni et al. *(143)* found an increase of chromosomal aberrations in workers from a factory where current exposure to styrene was high (9.6–46.4 ppm, 41–198 mg/m^3) but not in workers from another factory with low current exposure (0.4–4 ppm, 1.7–17 mg/m^3); the latter group of workers – who had experienced a high exposure to styrene in the past and had, therefore, a high cumulative exposure – showed an increase in chromosomal aberrations. Mäki-Paakkanen et al. *(120)* obtained a significant correlation between the number of cells with chromosomal aberrations and years of exposure. Tomanin et al. *(151)* could demonstrate an elevation of chromosomal aberrations in a high-exposure group (27–104 ppm, 115–443 mg/m^3) but not in a low-exposure group (5–24 ppm, 21–102 mg/m^3). Artuso et al. *(154)* obtained a significant styrene-exposure dependent trend for both chromosomal aberrations and SCEs among a high-dose group (gel coaters, laminators, rollers and assemblers; exposure

range 20–326 ppm, 85–1389 mg/m^3), a low-dose group (other tasks; exposure range 0.5–28 ppm, 2–119 mg/m^3), and controls.

Camurri et al. *(138)* observed a significant increase in SCEs in workers in reinforced plastics plants with mean airborne styrene levels of more than 47 ppm (200 mg/m^3) but not in plants with mean levels of 7–47 ppm (30–200 mg/m^3). Yager et al. *(149)* obtained a clear relationship among a group of boat manufacturers between SCE frequency and styrene exposure measured either as the concentration of styrene in workroom air (0.2–55 ppm, 0.9–234 mg/m^3; mean 15 ppm, 64 mg/m^3) or in exhaled air. Hallier et al. *(152)* observed an increase in SCEs among laminators exposed to high concentrations of styrene (approximately 40 ppm, 170 mg/m^3) but not in formers exposed to much lower levels (10 ppm, 43 mg/m^3). When hygienic and technical improvements at the workplace reduced the styrene exposure of the laminators to approximately 20 ppm (85 mg/m^3), a significant reduction in the SCE levels of the laminators was noted, although their values were still higher than in unexposed controls.

The few studies available on DNA strand breakage and *N*-acetylaminofluorene-induced unscheduled DNA synthesis in peripheral leukocytes have given positive results (Table 3). DNA strand breaks were shown to disappear quickly from the peripheral leukocytes following the exposure, so that blood samples collected before the work shift could be used as control samples *(122)*. Reinforced plastics workers showed elevated frequencies of GPA variant erythrocytes – a measure of mutations in the glycophorin A locus – with a significant effect especially among workers belonging to a high-exposure group (breathing zone styrene concentration ≥20 ppm, 85 mg/m^3) *(125)*. Another study also suggested an increase in GPA variant frequencies in a high-exposure group (average exposure concentration 32 ppm, 136 mg/m^3) as compared with a low exposure group (average exposure to 1.2 ppm, 5 mg/m^3 styrene), but final conclusions were complicated by inadequate matching of the groups *(124)*. Vodicka et al. *(119)* observed a weak elevation in the frequency of HPRT mutations in T-lymphocytes, while another study *(126)* was considered inconclusive.

In summary, genotoxic effects have been observed in the blood cells of reinforced plastics workers for var ious endpoints at styrene exposure levels of around 20–30 ppm (85–128 mg/m^3) and above. DNA breakage has been observed at exposure levels below 10 ppm. The role of styrene in generating the genotoxic effects seen in reinforced plastics workers is supported by the elevated levels of styrene-7,8-oxide DNA adducts observed in their peripheral leukocytes workers. Such adducts appear to be distinguishable following occupational exposures to only a low concentration of styrene.

Carcinogenic effects in humans

Cases of leukaemia and lymphoma were identified among workers exposed in the manufacture of styrene and polystyrene, and in the production or manufacture of styrene–butadiene *(13)*.

Epidemiological studies of styrene have been conducted in three types of industry: production of glass-reinforced plastics products, production of styrene monomers and styrene polymerization, and production of styrene–butadiene rubber. The malignancies observed in excess most frequently are of the lymphatic and haematopoietic system.

Reinforced plastics industry

In a European multinational study of more than 40 000 workers in the glass-reinforced plastics industry (660 plants), no overall excess of deaths from lymphatic and haematopoietic cancers was observed in comparison with national controls *(*159*)*. An increased risk for lymphatic and haematopoietic tumours was observed in Poisson regression models for years since first exposure ($P = 0.012$) and for average exposure ($P = 0.19$) but not for cumulative exposure. Within the models, there was an increasing trend in risk for lymphatic and haematopoietic cancer with average intensity of exposure, culminating in a relative risk (RR) of 3.6 (95% confidence interval (CI), 1.0–13) for the highest category, >200 ppm; for more than 20 years since first employment, the RR was 4.0 (95% CI, 1.3–12). Nonsignificant increases in risk with time since first exposure or cumulative exposure were noted for cancers of the pancreas, kidney, and oesophagus.

A study of cancer incidence in the reinforced plastics industry in Denmark involved 12 800 male workers who had been included within the above study and a further 24 000 workers with a lower probability of exposure to styrene. The mean annual air concentrations of styrene calculated for 128 of the companies studied ranged from 180 ppm (767 mg/m^3) in 1964–1970 to 43 ppm (183 mg/m^3) in 1976–1988. Within this cohort, there were 112 malignancies of the lymphatic and haematopoietic system, with 93.7 expected (standardized incidence ratio (SIR), 1.2; 95% CI, 0.98–1.4). In workers with more than 10 years since first employment, the SIR for leukaemia was 157 (107–222). The excess was concentrated mainly in those workers not previously included in the European study, in short-term workers with at least 10 years since first employment and in those employed before 1970 *(160)*. The same authors also reported the occurrence of deaths from solid cancers among the 36.610 reinforced plastics workers *(161)*. An increased incidence rate ratio (IRR) for pancreatic cancer was found (IRR 2.2, 95% CI, 1.1– 4.5).

In a large study on 5826 employees who had worked for at least 6 months between 1948 and 1977 in 30 reinforced plastics plants in the United States, no overall increase in risk for lymphatic and haematopoietic malignancies was observed *(162,163)*. The follow-up continued until the year 1989. The overall mortality rate was 108 (95% CI, 103–113), and the mortality rate from all cancers was 116 (95% CI, 105–127). Total lympho-haematopoietic cancers showed no excess. For workers involved in open-mould processing with high exposure to styrene, the standardized mortality ratio (SMR) for lymphatic and haematopoietic cancers was 141 (based on four cases). For the highest cumulative exposure (>100 ppm × years) and more than 20 years of latency, the SMR was 134 (5–373). Mortality for cancers at a number of sites was increased significantly. These included oesophagus (mortality rate 198; 95% CI, 105–322), bronchus, trachea, and lung (141; 120–164), cervix uteri (284; 136–521), and other female genital organs (202; 107–345). No positive dose–response relationship was found for the lympho-haematopoietic cancers or any other cancer in excess; IARC *(13)*, however, noted that the possibility that the two exposure variables included in the regression model were correlated may have reduced the likelihood of accurate assessment

A smaller study of 5021 employees in two reinforced plastics boat-building facilities in the United States showed no deaths from leukaemia or lymphomas (1.7 and 2.1 expected, respectively) *(164)*. 2060 individuals were considered to have had high exposure to styrene (mean levels of styrene in the air in two facilities, 42.5 and 71.7 ppm, 181 and 305 mg/m^3); 48% of them had worked only for one month to one year and only 5% for more than 5 years. In this group, no lymphatic or haematopoietic cancers were seen (about 1 expected).

A deficit of deaths from lymphatic and haematopoietic malignancies (6 observed, 14.9 expected) was reported in a cohort of 7949 men and women employed during 1947–1984 in eight companies in the United Kingdom manufacturing glass-reinforced plastics involving high exposure to styrene *(165)*. A nonsignificant excess was seen in deaths from cancers of the lung, pleura and mediastinum (SMR, 126; 95% CI, 94–166); this finding particularly related to workers who had had 1–9 years of exposure to styrene, but the risk did not increase with time from the first exposure. Follow-up of this cohort was later extended to 1990 as part of an international collaborative study *(159)*. By that time the previous deficit of lymphatic and haematopoietic cancer had largely disappeared (13 deaths; SMR, 88; 95% CI, 47–151) and the excess of lung cancer was less marked (77 deaths; SMR, 106; 95% CI, 84–132).

Styrene manufacture and polymerization

A study of chemical workers in the production of styrene and styrene derivatives in the United States found seven deaths from lymphatic and haematopoietic malignancies (except leukaemias) (SMR, 132; 95% CI, 58–272) and six from leukaemias (SMR 176; 95% CI, 64–383) *(166)*. In an update, a total of 28 deaths from lymphatic and haematopoietic cancer were recorded (SMR 144; 95% CI, 95–208) *(167)*.

A smaller United Kingdom study of 622 men exposed for at least a year in the production, polymerization and processing of styrene found an excess of deaths from lymphoma (3 observed, 0.56 expected) *(168)*.

Styrene–butadiene rubber production

The large cohorts of styrene–butadiene rubber workers showed increased risks for lymphatic and haematopoietic malignancies, but nested case–control analyses found little evidence for relationship to styrene *(169–171)*. The styrene exposures in these industries are at least one order of magnitude lower than in the reinforced plastics industry.

In summary, several epidemiological studies have suggested that workers exposed to styrene in the reinforced plastics industry have increased risk of lymphatic and haematopoietic tumours. The studies are not, however, fully conclusive because the observed associations are often based on small numbers, and in larger studies, dose relations are somewhat obscure.

Reproductive effects

The frequency of spontaneous abortions among women with definite or assumed exposure to styrene has been investigated in a number of studies; the majority do not indicate an increased risk in association with occupational exposure to styrene *(13)*. A study in Canada *(172)* found an increased risk for spontaneous abortions (18 observed; SMR, 158; 90% CI, 102–235) among women employed in polystyrene manufacture. The expected figures were derived from the experience of 47 316 pregnant women who had worked for 30 hours or more per week at the start of pregnancy. No styrene concentrations were given, and most of these women had had mixed exposures. Two studies in Finland found no increase in the frequency of spontaneous abortion or congenital malformation among the wives of men exposed occupationally to styrene *(173,174)*.

The conclusion thus remains that no clear association has been established between occupational exposure of either mothers or fathers to styrene and the frequency of spontaneous abortions or congenital malformations.

Evaluation of human health risks

Exposure evaluation

Concentrations of styrene in rural ambient air are generally less than 1 μg/m^3, while indoor air in such locations may contain several μg/m^3. Levels in polluted urban areas are generally less than 20 μg/m^3 but can be much higher in newly built houses containing styrene-based materials.

Health risk evaluation

Potentially critical effects for the derivation of a guideline for styrene are considered to be carcinogenicity/genotoxicity and neurological effects, including effects on development.

Styrene in its pure form has an odour detection threshold of 70 μg/m^3. Its pungent odour is recognized at concentrations three to four times greater than this threshold value.

The value of the available evidence for an association between exposure to styrene and small increases in lymphatic and haemotopoietic cancers observed in workers in some studies is limited by concurrent exposure to other substances, lack of specificity and absence of dose–response. In limited studies in animals, there is little evidence that styrene is carcinogenic. IARC has classified styrene in group 2B.

Styrene was genotoxic *in vivo* and *in vitro* following metabolic activation. In cytogenetic studies on peripheral lymphocytes of reinforced plastics workers, there were increased rates of chromosomal aberrations at mean levels of styrene of more than 120 mg/m^3 (>20 ppm). Elevated levels of single-strand breaks and styrene-7,8-oxide adducts in DNA and haemoglobin have also been observed. Although these genotoxic effects have been observed at relatively low concentrations, they were not considered as critical endpoints for development of a guideline, in view of the equivocal evidence of carcinogenicity for styrene.

The available data, although limited, indicate that neurotoxicity in the form of neurological developmental impairments, is among the most sensitive of endpoints. In the offspring of rats exposed to styrene at a concentration of 260 mg/m^3 (60 ppm), there were effects on biochemical parameters in the brain and behaviour.

Guidelines

Although genotoxic effects in humans have been observed at relatively low concentrations, they were not considered as critical endpoints for development of a guideline, in view of the equivocal evidence for the carcinogenicity of styrene.

In occupationally exposed populations, subtle effects such as reductions in visuomotor accuracy and verbal learning skills, and subclinical effects on colour vision have been observed at concentrations as low as 107–213 mg/m^3 (25–50 ppm). Taking the lower number of this range for precautionary reasons and adjusting this value in order to allow for conversion from an occupational to a continuous pattern of exposure (a factor of 4.2), and incorporating a factor of 10 for interindividual variation and 10 for use of a lowest-observed-adverse-effect level (LOAEL) rather than a no-observed-adverse-effect level (NOAEL) this results in a guideline of 0.26 mg/m^3(weekly average). This value should also be protective for the developmental neurological effects observed in animal species.

The air quality guideline could also be based on the odour threshold. In that case, the peak concentration of styrene in air should be kept below the odour detection threshold level of 70 $\mu g/m^3$ as a 30-minute average.

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