Environmental Health Criteria 26

STYRENE

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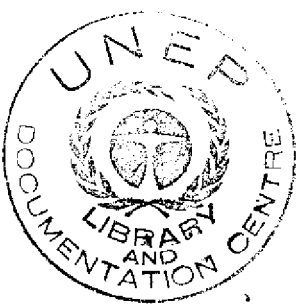

World Health Organization
Geneva, 1983

The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluation of the conclusions contained in the criteria documents.

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ENVIRONMENTAL HEALTH CRITERIA FOR STYRENE

Further to the recommendations of the Stockholm United Nations Conference on the Human Environment in 1972, and in response to a number of World Health Assembly resolutions (WHA23.60, WHA24.47, WHA25.58, WHA26.68) and the recommendation of the Governing Council of the United Nations Environment Programme, (UNEP/GC/10, July 3 1973), a programme on the integrated assessment of the health effects of environmental pollution was initiated in 1973. The programme, known as the WHO Environmental Health Criteria Programme, has been implemented with the support of the Environment Fund of the United Nations Environment Programme. In 1980, the Environmental Health Criteria Programme was incorporated into the International Programme on Chemical Safety (IPCS). The result of the Environmental Health Criteria Programme is a series of criteria documents.

The Institute of Occupational Health (Director, Dr J. Rantanen), Helsinki, was responsible, as a Lead Institution of the IPCS, for the preparation of the first and second drafts of the Environmental Health Criteria document on styrene. Dr H. Vainio co-ordinated the work.

The Task Group for the Environmental Health Criteria for Styrene met in Helsinki from 8 to 15 November, 1982. The meeting was opened by Dr J. Rantanen and Dr F. Valic welcomed the participants and representatives of other organizations on behalf of the three organizations co-sponsoring the IPCS (UNEP/ILO/WHO). The Task Group reviewed and revised the second draft criteria document and made an evaluation of the health risks of exposure to styrene.

The efforts of all who helped in the preparation and the finalization of the document are gratefully acknowledged.

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Partial financial support for the publication of this criteria document was kindly provided by the United States Department of Health and Human Services, through a contract from the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA - a WHO Collaborating Centre for Environmental Health Effects.

1 SUMMARY AND RECOMMENDATIONS FOR FURTHER STUDIES

1.1 Summary

1.1.1 Uses and sources of exposure

Styrene (ethenylbenzene) is a commercially important chemical used in the production of polymers, copolymers, and reinforced plastics. Exposure mainly occurs in industries and operations using styrene, and industrial sources are the most likely cause of general population exposure. Other potential sources of general population exposure include motor vehicle exhaust, tobacco smoke, and other combustion/pyrolysis processes. Low-level exposure of the general population can occur through the ingestion of food products packaged in polystyrene containers.

General population exposure levels are usually orders of magnitude lower than occupational exposure levels - though the latter vary considerably depending on the operations concerned. While some exposure occurs in styrene/polystyrene manufacturing plants, the highest levels of exposure are found in the industries and operations concerned with the fabrication and application of plastics. Thus, industrial processes, such as those in the reinforced plastics industry, require the greatest attention. In addition, clean-up and maintenance procedures in many related industries may result in significant exposures.

1.1.2 Chemobiokinetics, biotransformation, and biological monitoring

The results of controlled laboratory studies on animals and human beings have shown that uptake of styrene is rapid and that it is widely distributed throughout the body. Uptake is mainly via the pulmonary and, to a lesser extent, the dermal and oral routes.

Styrene is distributed through the whole body and stored in lipid depots. Its subsequent slow elimination from the tissue indicates a potential for bioaccumulation following repeated daily exposure.

Styrene is biotransformed largely via the 7,8-epoxide by the mixed function oxidase system. The principal urinary metabolites are α-hydroxybenzeneacetic (mandelic) and phenylglyoxylic acids. Recent evidence suggests that the pattern of urinary metabolite excretion varies with mammalian species. Other minor metabolic pathways may also be important in the toxicological assessment of this compound.

The elimination of styrene and its metabolites appears to involve a two-compartment kinetic model that becomes

monophasic in experimental animals at high exposure levels. This suggests the existence of saturable metabolic pathways.

Exposure levels can be assessed by the quantitative analysis of alveolar air or determination of the urinary metabolites, mandelic and phenylglyoxylic acid. At present, urinary mandelic acid appears to be the most reliable biological indicator of exposure in human beings.

1.1.3 Adverse health effects

1.1.3.1 Acute effects

Exposure levels of 420 mg/m^3 (100 ppm) and above cause irritation of the mucous membranes of the eyes and the upper respiratory tract in man. Similar effects have been observed in experimental animals. Exposure of human volunteers to levels exceeding 840 mg/m^3 (200 ppm) resulted in drowsiness, nausea, and disturbed balance, within a few minutes. Prolonged reaction times have been reported in connection with short-term exposure of human volunteers to 840 mg/m^3 (200 ppm).

1.1.3.2 Nervous system effects

Epidemiological studies on workers with long-term occupational exposure to styrene have shown an increased prevalence of abnormal EEGs associated with urinary mandelic acid concentrations of 700 mg/litre or more; at 1600 mg/litre, a reduction in psychomotor performance and visuomotor accuracy in psychological tests has been observed. However, definitive evidence of adverse effects on the peripheral nervous system is still not available.

1.1.3.3 Genetic effects

When metabolically activated, styrene may be mutagenic and clastogenic in many experimental systems. Conflicting results obtained in some *in vitro* mutagenicity assays, were presumably due to differences in metabolic activation and inactivation of styrene. Styrene 7,8-oxide is the main reactive intermediate of styrene biotransformation. It is an alkylating agent that is mutagenic and clastogenic in many *in vitro* test systems.

Several studies have indicated an increased frequency of structural chromosome aberrations in the peripheral blood lymphocytes of workers exposed to styrene in the reinforced plastics industry. Negative results have been reported in workers employed in the production of styrene monomer and polystyrene, where exposure to styrene is lower.

Because of the complexity of the evaluation of the total exposure of workers in the reinforced plastics industry, it has not been possible to show, unequivocally, that styrene is the cause of the observed somatic chromosome aberrations. Even though the biological and health significance of somatic chromosome damage is not understood at present, the increase in such effects is considered to be an indicator of possible adverse health effects.

1.1.3.4 Carcinogenic effects

Several case reports and epidemiological studies have implied an increased risk of cancers of the lymphatic and haematopoietic systems in workers involved in the manufacture of styrene and polystyrene and styrene-butadiene rubber. However, at present, there is insufficient evidence to establish a direct cause and effect relationship between styrene exposure and cancer in human beings.

A single experimental study in mice indicated limited evidence for the carcinogenicity of styrene in this species. Orally administered styrene 7,8-oxide, the primary metabolite of styrene, was carcinogenic in rats.

1.1.3.5 Effects on reproduction

Results of a few studies on mammals (rats, mice, and Chinese hamsters) suggest that inhaled styrene has embryotoxic effects. Only a limited number of studies on women are available, with inconclusive results.

1.2 Recommendations for Further Studies

Technological studies are needed to develop methods of resin production with reduced exposure of workers.

Further elucidation of urinary metabolite excretion kinetics and their relationship with styrene exposure is recommended.

Morphological studies of the effects of styrene on the central and peripheral nervous systems in experimental animals are needed. Well-conducted human epidemiological investigations, with monitored individual exposure levels, are necessary to evaluate nervous system effects and should include neurophysiological and psychological tests.

Studies on the genetic effects of styrene should be aimed at defining the relationship between dose and the induction of chromosome damage in workers with various types of occupational styrene exposure. More experimental studies on styrene are needed to verify the suspected role of styrene as a genetic hazard in the occupational environment.

Research on the health effects of styrene should primarily be devoted to resolving the issue of carcinogenesis both in experimental animal studies and in epidemiological studies on styrene-exposed workers, particularly in industries with high styrene exposure.

The effects of styrene on the endocrine system and reproduction should continue to be evaluated by means of experimental animal studies and epidemiological investigations.

Further validation of experimental methods and analytical techniques should be undertaken as well as the establishment of quality control programmes.

2. PROPERTIES AND ANALYTICAL METHODS

Chemical and physical properties of styrene [a]

Chem. Abstr. Services Reg. No.: 100-42-5

Chem. Abstr. Name: Ethenylbenzene

Synonyms: phenethylene; phenylethene; phenylethylene; styrol; styrole; styrolene; vinylbenzene; vinylbenzol; cinnamene; cinnamol

Molecular formula: C_8H_8

Relative molecular mass: 104.14

Description: Colourless, viscous liquid with a pungent odour

Boiling point: 145.2°C; 33.6°C at 1.33 kPa (10 mmHg)

Freezing point: - 30.63°C

Density: (d_4^{20}) 0.9060

Refractive index: (n_D^{20}) 1.5468

Spectroscopy data: λ_{max} 245.3 (E_1^1 = 1514)

Solubility: slightly soluble in water (30 mg/100 ml at 20°C); soluble in ethanol, diethylether and acetone; very soluble in benzene and petroleum ether

Volatility: Vapour pressure: 0.8666 kPa (6.45 mmHg) at 25°C; 1.33 kPa (10.0 mmHg) at 35°C

Stability: Flash-point, 31°C; polymerizes easily at room temperature in the presence of oxygen and oxidizes on exposure to light and air: must be stored in an inert atmosphere or with added inhibitors (e.g., 0.001% tertiary butylcatechol)

Autoignition temperature: 490°C

Inflammability range (20°C): 1.1-6.1%

Vapour density: (air = 1) 3.6

Conversion factors: 1 ppm in air = 4.2 mg/m³;
1 mg/m³ in air = 0.24 ppm

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[a] Chemical and physical properties of the pure substance; from Foerst (1965), IARC (1979), Iti (1975), and Weast & Astle (1981).

2.1 Analytical Procedures

Various methods are available for measuring styrene and styrene metabolites in environmental media and biological fluids and tissues.

A number of laboratories have shown the reproducibility of analytical results. However, there have not been any systematic interlaboratory analysis comparison programmes.

2.1.1 Measurement of styrene in air

Several methods have been developed for the measurement of styrene vapour in the working environment.

Sampling can be carried out by means of absorption in a solvent such as ethanol (Yamamoto & Cook, 1968; Leithe, 1971); adsorption on a solid material such as activated carbon (NIOSH, 1974; Kalliokoski & Pfäffli, 1975; Burnett, 1976;) or porous polymer beads (Dietrich et al., 1978) and by grab sampling either into empty tubes (White et al., 1970) or with a motor-driven syringe (Bergman, 1977). Passive monitoring badges can also be used for sampling (Ikeda et al., 1982). Styrene is recovered from adsorption tubes by solvent desorption (NIOSH, 1974; Kalliokoski & Pfäffli, 1975) or heating (Dietrich et al., 1978). Samples taken by a syringe require prompt analysis. Continuous monitoring of the airborne styrene concentration is possible using an infrared spectrometer equipped with a gas cell and pump.

Adsorption tubes have replaced absorption bottles to a great extent in sampling. They can be incorporated into small, portable sampling devices that do not contain liquids and are well adapted for either personnel or area monitoring. Activated carbon is the most widely used solid adsorbent (Dietrich et al., 1978). Though styrene polymerizes easily, samples of styrene on activated carbon are stable, even when exposed to moderate heat and sunlight (Saalwaechter et al., 1977). In the standard method of NIOSH (1974), the styrene is desorbed from the activated carbon tubes with carbon disulfide, which offers the advantage that the solvent peak is insignificant when a flame ionization detector is used (McNair & Bonelli, 1968). The desorption procedure does not remove all the styrene and the results must be corrected for desorption efficiency. According to Burnett (1976), the average desorption efficiency of carbon disulfide for styrene is 85%. The desorption efficiency varies somewhat from one batch of activated carbon to another. It is therefore advisable to determine the desorption efficiency for each batch of activated carbon or commercial tubes (NIOSH, 1974). If activated carbon is also added to the standards, the

desorption efficiency correction is not needed (Kalliokoski & Pfäffli, 1975). Choi & Fung (1979) recommended the use of an internal standard (ethylbenzene) to avoid the effects caused by changes in the instrumental conditions. In addition to carbon disulfide, other solvents, such as dimethyl formamide (Kalliokoski & Pfäffli, 1975) or cyclohexanone (Saalwaechter et al., 1977) can be used for desorption.

The use of porous polymer tubes, such as Tenax GC (Dietrich et al., 1978), with subsequent thermal desorption and gas chromatographic analysis offers the advantage that the entire sample can be analysed at one time and in the absence of the solvent peak. Commercial instrumentation for thermal desorption is available. Choi & Fung (1979) warn of the partial polymerization of styrene during thermal desorption. When styrene is determined by UV-spectrometry, wavelengths of 245 and 290 nm can be used. The former wavelength gives stronger absorption but is more sensitive to interferences (Manson et al., 1965; Yamamoto & Cook, 1968; Babina, 1969; Leithe, 1971).

Analysis of samples can be made by visible, ultraviolet, and infrared spectrophotometry, or gas chromatography (Fishbein, 1979). The direct determination of styrene in air without a preceeding concentration step is possible using IR-spectrometry or gas chromatography. Commercial variable-path, IR analysers are available. The recommended wavelengths are 11.0 and 13.0 µm (Thompson, 1974). Styrene also has absorption bands at 3.3, 6.7, 10.2, and 14.8 µm. The major absorption bands of the most usual interfering substance, acetone, are at 3.4, 5.8, 7.4, and 8.2 µm (Wilti, 1970); also acetone has minor bands at 11.0 and 13.0 µm (the latter is weaker). The band with the least interference is 14.8 µm. However, the interference does not prevent the use of the other bands; in fact, it may be advantageous to perform a multicomponent analysis by repeating the measurement using different wavelengths. Commercial microprocessor control devices are available with which it is possible to carry out automatic, simultaneous multi-point monitoring of several air pollutants (Jacobs & Syrjala, 1978). The lowest detectable concentration of styrene is about 1 mg/m³ (0.2 ppm), in the absence of interference, using 11.0 µm as the analytical wavelength with a 20 m cell.

Gas chromatographic analysis with a flame ionization detector is the most sensitive analytical method for styrene. Several column stationary phases are suitable including (10%) FFAP (NIOSH, 1974), (15%) Carbowax 1540 (Gotell et al., 1972), (10%) XF 1150 (Kalliokoski & Pfäffli, 1975), and (5%) SE-30 (Bergman, 1977). The limit of detection, which varies according to the gas chromatographic conditions, is about 0.1 mg/m³ (0.02 ppm) for direct air analysis (Stewart et al.,

1968) and 0.05-0.1 mg for desorbed sample injections (NIOSH 1974; Kalliokoski & Pfäffli, 1975). The coefficient of variation for gas chromatographic analysis of solutions containing 0.1-1 mg styrene in 1 ml of solvent is about 2%.

2.1.2 Measurement of styrene and styrene metabolites in biological samples

Styrene has been measured in blood, exhaled air, and subcutaneous fat. The concentration of styrene in blood and exhaled air falls rapidly during the first hour following termination of exposure. Thus, a single sample of exhaled air or blood is of limited use. A number of samples are required for the establishment of a kinetic "decay curve" from which the assessment of a whole day's exposure may be possible (Stewart et al., 1968; Fernandez & Caperos, 1977; Fields & Horstman, 1979).

2.1.2.1 Exhaled air

Gas chromatography is the method of choice for the rapid determination of styrene concentrations in exhaled air (Gotell et al., 1972; Fernandéz & Caperos, 1977). The detection limit of the method is 0.04 mg/m^3 (0.01 ppm) (Fernandez & Caperos, 1977). The limitation of sample collection in polyethylene bags is the instability of the sample, due to polymerization of the styrene within the bag, after sampling (Fields & Horstman, 1979). Brooks et al. (1980) have developed an improved method for the measurement of styrene in exhaled air, with increased stability of samples as well as reliability of sample collection. For sampling, they used a bubbler system instead of polyethylene bags and were able to reduce the detection limit to 0.02 mg/m^3 (0.005 ppm).

2.1.2.2 Blood

Styrene concentrations in venous blood have been determined by gas chromatography using the "head space" technique (Åstrand et al., 1974; Schaller et al., 1976; Withey & Collins, 1977; Benčev & Rizov, 1981) or after previous extraction (Stewart et al., 1968; Brooks et al., 1980). Wolff et al. (1978) have developed a direct spectrofluorometric method for the determination of styrene in blood with a very low reported detection limit.

2.1.2.3 Subcutaneous adipose tissue

Estimation of styrene concentrations in adipose tissue possible in selected cases using a non-surgical, needle biop

technique to obtain the tissue sample. After extraction, the styrene absorbed in the adipose tissue can be determined easily using gas chromatographic methods (Savolainen & Pfäffli, 1977; Wolff et al., 1977). The detection limit for a 10 mg sample is 40 µg styrene/kg adipose tissue (Wolff et al., 1977) with a recovery of 98 ± 1.9% (Savolainen & Pfäffli, 1977). Determination of styrene in adipose tissue has also been performed using a modified "headspace" gas chromatographic technique (Engström et al., 1978a).

2.1.2.4 Urine

Evaluation of styrene exposure may be based on the urine concentration of phenylglyoxylic acid (Ohtsuji & Ikeda, 1970; Götell et al., 1972; Härkönen et al., 1974), on the combined concentrations of mandelic and phenylglyoxylic acids (Guillemin & Bauer, 1978; Elia et al., 1980), or on the ratio of mandelic to phenylglyoxylic acid (Philippe et al., 1974). Most of the methods for the determination of mandelic and phenylglyoxylic acid in urine are based on gas chromatographic techniques (Slob, 1973; Buchet et al., 1974; Engström & Rantanen, 1974; Guillemin & Bauer, 1976; Flek & Sedivec, 1980). Derivatization of the acids is necessary before the gas chromatographic analysis and both methylation (Buchet et al., 1974; Flek & Sedivec, 1980) and silylation (Slob, 1973; Engström & Rantanen, 1974) have been used for this purpose. Gas chromatographic determination of mandelic acid is reliable and simple, whereas the determination of phenylglyoxylic acid involves problems of derivatization, that can only be overcome by special techniques (Bauer & Guillemin, 1976; Flek & Sedivec, 1980). Detection limits ranging from 0.6 to 6 mg/litre and variation coefficients ranging from 1 to 3% have been reported for the different gas chromatographic procedures (Slob, 1973; Engström & Rantanen, 1974; Flek & Sedivec, 1980).

Determination of mandelic and phenylglyoxylic acid without derivatization can be performed using methods based on isotachoforesis (Sollenberg & Baldesten, 1977) and high performance liquid chromatography (Ogata & Sugihara, 1978; Poggi et al., 1982).

Instability of samples may be a problem in urine analysis for mandelic and phenylglyoxylic acid, because both acids are prone to change during storage at room temperature (Flek & Sedivec, 1980). This is especially the case with phenylglyoxylic acid, which should not be stored for periods longer than 24 h at room temperature; freezer storage is recommended.

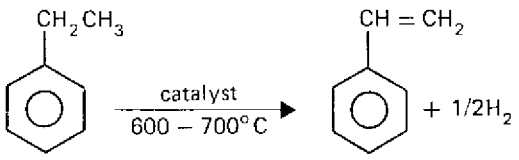
3. SOURCES OF STYRENE IN THE ENVIRONMENT

3.1 Production of Styrene

The first step in styrene production is the catalytic alkylation of benzene with ethylene, both raw materials being supplied primarily from the petroleum industries. The following two processes are in use for the production of styrene (US EPA, 1980).

(a) Dehydrogenation of ethylbenzene

Ethylbenzene is dehydrogenated to styrene by the following reaction:



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This is the desired selective reaction; however, various non-selective reactions will occur. For example, a reactor effluent contains an average of 0.7% benzene and 1.0% toluene (Faith et al., 1975).

The crude styrene, with an average composition of 37% styrene, 61% ethylbenzene, 1.0% toluene, 0.7% benzene, and 0.3% tars, is passed through a pot containing sulfur or some other polymerization inhibitor and is then fed into a vacuum column system. The overhead from a primary fractionating column is fractionated to separate the ethylbenzene, which is recycled, from the benzene and toluene, which are separated by distillation. The bottoms from a primary fractionating column are distilled to obtain the pure styrene product (US EPA, 1980).

(b) Co-product with propylene oxide

In late 1977, production began in a plant that makes styrene by the following method (Soder, 1977):

$$C_6H_5CH_2CH_3 + O_2 \longrightarrow C_6H_5CHOOHCH_3$$

(ethylbenzene) (ethylbenzene hydroperoxide)

$$C_6H_5CHOOHCH_3 + CH_3CH{=}CH_2 \longrightarrow C_6H_5CHOHCH_3 + CH_3\overset{O}{\overset{\diagdown\diagup}{C}}HCH_2$$

(propylene) (methyl phenyl carbinol) (propylene oxide)

$$C_6H_5CHOHCH_3 \longrightarrow C_6H_5CH{=}CH_2 + H_2O$$

(styrene)

Ethylbenzene is oxidized to the hydroperoxide, which is then reacted with propylene to yield the propylene oxide and a co-product, methyl phenyl carbinol. The carbinol is then dehydrated to styrene (US EPA, 1980). Expected impurities may include propylbenzene, isopropylbenzene, and α-methylstyrene.

Table 1 shows the annual production of styrene monomer in the world. The Federal Republic of Germany, Japan, and the USA are the major producers (Tossavainen, 1978). It is estimated that the world styrene consumption will grow at an average of 5.1% per year in the period 1982-90 reaching 13.6 million tonnes by 1990 (Chemical Market Review, 1981).

Table 1. Production of styrene [a]

Country	Year	Production (tonnes)
Canada	1974	146 000
France	1976	270 000
Germany, Federal Republic of	1976	860 000
Italy	1976	325 000
Japan	1976	1 090 000
Mexico	1974	30 000
Spain	1976	60 000
USA	1976	2 864 000
Others	1976	approx. 1 000 000
World	1977	7 000 000

[a] From: Tossavainen (1978).

3.2 Uses

The uses of styrene (monomer) in the USA in 1980 are listed in Table 2. The pattern of consumption is essentially the same elsewhere in the world including Europe and Japan (Tossavainen, 1978) and no significant change is foreseen as far as 1990 (European Chemical News, 1982).

3.3 Main Sources of Environmental Pollution

According to Pervier et al. (1974), the major sources of styrene contamination of the environment are the petrochemical industries. Emissions from styrene production may result from vents on distillation columns and other processing equipment, storage tank losses, miscellaneous leaks

Table 2. Use pattern of styrene in the USA in 1980

Material	%
polystyrene	45
acrylonitrile-butadiene-styrene plus styrene-acrylonitrile resin	10
styrene-butadiene rubber	8
styrene-butadiene latex	6
unsaturated polyester	5
miscellaneous	4
exports	17

and spills, waste-waters, and solid process wastes. However, losses from production are low in comparison with other petrochemical losses. Some styrene can also be emitted from polymerization processes.

Styrene can be released into the environment during various disposal procedures, e.g., during the incineration of many types of styrene polymers. Styrene has been detected in hydrocarbon exhausts from spark-ignition engines (Flemming, 1970), in oxyacetylene and oxyethylene flames (Crittenden & Long, 1976), and in cigarette smoke (section 4.1.1). The domestic use of polyester resins has increased potential exposure patterns. Exposure may occur when styrene is used as a solvent during the preparation of resin flooring (Gadalina et al., 1969; Kaznina, 1969); or through the use of styrene in various hobbies, crafts, and toys (Smirnova & Yatakova, 1966).

4. ENVIRONMENTAL EXPOSURE LEVELS

4.1 General Environment

Some data are available on styrene concentrations in air, cigarette smoke, water, and food. However, they are not sufficiently systematic to provide an overall picture and an estimate of the main routes of human exposure.

Some of the following data for styrene levels in air and water are drawn from a survey of literature, released in 1980 by the US Environmental Protection Agency (US EPA, 1980), concerning exposure to several potential environmental contaminants.

4.1.1 Ambient air

Styrene has been detected in ambient air in a wide variety of locations.

In a study to develop procedures for determining specific contaminants, Neligan et al. (1965) measured the amounts of a number of hydrocarbons in the urban atmosphere in the USA. The results of analysis of air from southern California in 1965 showed styrene to be present at levels of 0.008-0.063 mg/m^3 (0.002-0.015 ppm) with an average level of 0.021 mg/m^3 (0.005 ppm).

Styrene has also been found in the urban atmosphere in Japan, (Hoshika, 1977) at levels of about 0.0008 $\mu g/m^3$ (0.0002 ppm). Valenta (1966) measured the air and water contamination near a butadiene-styrene rubber plant in Czechoslovakia. One of the main sources of contamination was the plant warehouse for liquid materials. A styrene concentration of 0.07 mg/m^3 (0.017 ppm) was found in the immediate downwind vicinity of the warehouse, compared with a concentration of 0.03 mg/m^3 (0.007 ppm) at a distance of 800 m.

Schofield (1974) analysed hydrocarbon emissions from vehicles and found that 0.76% of total hydrocarbons was in the form of styrene in the exhaust of conventional engines, and 2.67% in the exhaust of rotary engines. Styrene has also been identified in cigarette smoke, reported levels ranging from 18 to 48 µg per cigarette (Johnstone et al., 1962; Baggett et al., 1974; Jermini et al., 1976).

4.1.2 Water

Styrene, detected in drinking water (US EPA, 1980) and in river water (Rosén et al., 1963; Gordon & Goodly, 1971; Bertsch et al., 1975), was usually traceable to an industrial source or to improper disposal. An example of serious

contamination of a water supply through improper disposal of styrene was reported by Grossman (1970) in which well water close to the site where two drums of styrene had been buried was found to have a disagreeable odour and contained a styrene concentration of 0.1-0.2 mg/litre. Water from another well close to the site of a waste dump from a styrene-butadiene plant contained a styrene concentration of 0.01-0.02 mg/litre (Valenta, 1966).

It seems that while styrene can be detected in water, it is not one of the frequent contaminants, nor is it present in large amounts.

4.1.3 Food

Polystyrene and its copolymers such as acrylonitrile-butadiene-styrene (ABS), have been widely used as food packaging materials. Currently available analytical surveys of food and food packaging have shown that the styrene monomer migrates into food from both rigid and expanded polystyrene foam containers. According to Withey & Collins (1978), the lowest concentration of monomer in rigid containers was 700 ppm and, the highest was 3300 ppm. Other studies give figures of a similar order of magnitude (Hamidullin et al., 1968). The lowest concentration of monomer in expanded polystyrene foam was 87 ppm (Withey, 1976). The highest migration figure (245.5 ppb) was found in samples of sour cream contained in rigid polystyrene containers (Withey & Collins, 1978). Styrene leached from containers at 0.2-0.5 ppm conveyed a disagreeable odour and taste to dairy products (Jensen, 1972). However, styrene was not detected in milk stored in polystyrene containers for up to 8 days (detection limit 50 ppb) (Finley & White, 1967). In another study, styrene rates of leaching ranged from 0.0077, 0.0078, and 0.0078 $\mu g/cm^2$ respectively, for foam cups containing water, tea, and coffee, to 0.036, 0.064, and 0.210 $\mu g/cm^2$, respectively for foam, impact, and crystal polystyrene cups containing 8% ethanol (Varner & Breder, 1981a,b).

In general, styrene concentrations in food are between 3 and 4 orders of magnitude lower than that in the package. It appears that the contribution to the total body burden of styrene via food is minimal.

4.2 Work Environment

Occupational exposure to styrene can be classified according to the types of operations in which it is present. In polystyrene manufacture, occupational chemical exposure is mainly to styrene. In reinforced plastics applications, where styrene is a solvent-reactant for copolymerization, styrene is

also the major air contaminant; however, there may be concomitant exposure to glass fibres, catalysts, accelerators, cleaning agents, and other chemicals. During styrene-butadiene rubber production, workers may also be exposed to such chemical substances as benzene, butadiene, carbon disulfide, and trichloroethylene and, in factories that produce styrene, there may be additional exposure to benzene and ethyl benzene. In many of the applications, the operations involve potential skin contact with liquid styrene.

4.2.1 Styrene and polystyrene manufacture

Concentrations of styrene recorded in a number of factories producing styrene or polystyrene are summarized in Table 3.

According to available reports, the styrene concentrations found in polystyrene production are generally less than 21 mg/m^3 (5 ppm); though occasional values of 210 mg/m^3 (50 ppm) or more have been reported.

Table 3. Concentration of styrene in air of polystyrene manufacturing plants

Year	Authors	Exposure levels mg/m^3 (ppm)	Work area/process
1952	Barsotti et al.	800 (192)	loading into polymerization tower
1963	Zlobina et al.	2-9 (0.5-2)	block production
		4-9 (1-2)	emulsion
		< 50 (< 12)	cleaning
1972	Ponomareva & Zlobina	10 (2)	polymerization
		< 5 (< 1)	polystyrene filament production
		< 5 (< 1)	short & intermittent operation
		0.47-0.68 (0.11-0.16)	drying & packaging
1974	Maier et al.[a]	< 21 (< 5)	at breathing zone of workers (involved in routine operations)
1978	Wolff et al.[a]	(11 samples were between 21-378mg/m^3 (5-90 ppm)	
1979	Thiess & Friedheim	244 (58)	technical services operation
		4-50 (1-12)	laboratory operation

[a] These investigators also identified benzene, ethylbenzene, toluene, acetone. The highest measured concentrations of these solvents, including styrene, resulted from spills and leaks.

Table 4. Concentration of styrene in air of reinforced plastics plants

Year	Author(s)	No. of plants	Exposure levels mg/m³ (ppm)	Work area/process
1967	Huzl et al.	5	210-420 (50-100)	styrene containing polyester resin applied by hand in 4 plants and sprayed in 1
1960	Bardodej et al.	2	63-840 (15-200)	polyester resin applied by hand
1964	Zielhuis et al.	3	101-378 (24-90) ≈ 29 (≈ 7)	manufacturing of boats, automobile bodies production of small objects and upholstery; laboratory
1966	Simko et al.	3	21-189 (5-195)	manufacture of reinforced plastics
1968	Matsushita et al.	1	> 2520 (> 600)	lamination of plywood with styrene-containing resin
1972	Gotell et al.	4	84-1218 (20-290)	production of reinforced plastics
1974	Bodnei et al. (1973-1974)[a]	1	189-2130(45-550)	reinforced plastics and bathtub manufacturing
1976	Kallioski	22	84-1260 (20-300)	boat laminating and other reinforced plastics manufacturing
1977	Rosensteel et al. (1975-1976)[a]	1	147-462 (35-110)	reinforced plastic boat plant (peak conc. of 1260-1680mg/m³ (300-400 ppm) for approx. 5 min)
1977	Bergman	4	42-714 (10-170)	Hand layup & spraying in the reinforced plastic boat industry (peak conc. of 256-2146 mg/m³ (61-511 ppm) for approx. 60 min)
1979	Brooks et al. (1977)[a]	3	168-966 (40-230)	
1979	Kjellberg et al.	1	13-59 (3-14)	boat fabrication
1981	Crandall (1978-1979)[a]	7	8-756 (2-180)	hull, deck & small part lamination and gelcoating process
1981	Schumacher et al.	12	< 1260 (< 300)	Table 7
1982	Ikeda et al.	5	< 4-1075 (< 1-256)	reinforced plastic boat plants

[a] Years when the study was conducted.

4.2.2 Reinforced plastics applications

Data concerning air levels of styrene in reinforced plastics plants, presented in Table 4, demonstrate that higher air levels of styrene are monitored in this industry than in styrene polymerization processes.

Tables 5, 6, 7, and 8 are designed to provide additional information, useful in the assessment of occupational exposure and to complement data presented in Table 4.

The resin manufacturers have tried to decrease styrene emissions by reducing the styrene content of resins and by using thixotropic resins and film-forming additives. They (Nylander 1979; Synthetic Resins Limited, 1980; Voskamp & Studenberg, 1981) claim that significant decreases in styrene emission can be achieved using these new resins, often called low styrene emission resins (LSE resins). In the study of Schumacher et al. (1981), the results of laboratory tests on such resins suggested a 30% reduction in styrene vapour, but when tested in the workplace there was essentially no difference in the exposure levels of the workers and the authors suggested the need for further studies to record any effects from the use of the new resins.

Concentrations of styrene found during the production of reinforced plastics (Zielhuis et al., 1964; Götell et al., 1972; Bodnei et al., 1974; Rosensteel & Meyer, 1977) were generally much higher than those found in the polystyrene production plants, with peak concentrations as high as 6300 mg/m^3 (1500 ppm). Most of the environmental concentrations of styrene in reinforced plastic plants, summarized in Table 4, are 8-h time-weighted-average (TWA) concentrations, but some values represent shorter periods. It can be seen from the table that exposure occurs not only in hand layup and spraying operations using open moulds, but also in mechanical and closed mould work. Furthermore, workers who do not handle the polyester resin themselves but work in the laminating room undergo passive exposure. It should be noted that the sampling strategy used influences the results; for example, breathing zone concentrations may differ considerably from the general air concentrations.

4.2.3 Styrene-butadiene applications

Hazards encountered in the synthetic rubber industry during the Second World War were discussed by Wilson (1944). The principal chemicals used in the manufacture of rubber, at that time, were styrene, butadiene, and acrylonitrile.

More recently, other studies of synthetic rubber manufacture have shown that levels of styrene are low.

Table 5. Airborne styrene concentrations at 36 Finnish factories using hand layup method [a]

(i) Mean 8-h TWA breathing zone styrene concentrations at 22 factories

Factory	mg/m³	ppm	Factory	mg/m³	ppm
1	1109	264	12	223	53
2	995	237	13	214	51
3	836	199	14	197	47
4	613	146	15	193	46
5	433	103	16	189	45
6	382	91	17	185	44
7	382	91	18	169	38
8	340	81	19	134	32
9	336	80	20	134	32
10	294	70	21	126	30
11	273	65	22	97	23

(ii) Mean breathing zone styrene concentrations during shorter (1-5 h) industrial hygiene surveys at 14 factories

Factory	mg/m³	ppm	Factory	mg/m³	ppm
23	1029	244	30	416	99
24	819	195	31	302	72
25	50	12	32	235	56
26	769	183	33	193	46
27	622	148	34	105	25
28	521	124	35	92	22
29	491	117	36	67	16

(iii) Distribution of styrene concentrations by product type

Product type	8-h TWA styrene conc. (factories 1-22)		TWA styrene conc. for periods of 1-5h (factories 23-36)	
	mg/m³	ppm	mg/m³	ppm
Boats	483	115	865	206
Other products				
- small objects	210	50	424	101
- large objects	357	85	248	59

[a] Adapted from: Kallioski (1976).

Baširov (1968) reported concentrations of 60-130 mg/m³ (14-31 ppm) in a 1968 study of a synthetic rubber plant. In a health risk evaluation in a similar type of plant, Gunter & Lucas (1973) were unable to find any measurable concentrations of styrene. However, styrene levels varying between 42 and 840 mg/m³ (10 and 200 ppm) were measured in a synthetic rubber plant by Spinazzola et al. (1980).

4.2.4 Summary of occupational exposure

In summary, occupational exposure to styrene varies considerably depending on the operations concerned. While styrene is present in detectable amounts in styrene/polystyrene manufacturing plants, the greatest exposures occur in the operations and industries that use unsaturated polyester resins dissolved in styrene. However, in all industrial operations using styrene, high levels of exposures occur during the clean-up and maintenance procedures.

Table 6. Airborne styrene concentrations at a factory in the USA [a]

Job category	8-h TWA styrene exposure (mean ± SD) mg/m³	(ppm)
Prefabrication	11.8 ± 16.8	(2.8 ± 4)
Gel coating	288 ± 251	(68.5 ± 59.7)
Hand layup	350 ± 179	(83.4 ± 42.7)
Hand layup, other areas	108 ± 110	(25.7 ± 26.2)
Woodwork/upholstery	14.3 ± 8.8	(3.4 ± 2.1)
Final assembly	14.3 ± 8.8	(3.4 ± 2.1)
Custom moulding	27.3 ± 16.4	(6.5 ± 3.9)
Small boat	17.2 ± 4.2	(4.1 ± 1.0)
Miscellaneous	16.4 ± 13.0	(3.9 ± 3.1)

[a] Adapted from: Brooks et al. (1979).

Table 7. Airborne styrene concentrations at 12 factories in the USA. The figures in the columns represent the number of workers in each exposure group [a]

Classification	Styrene concentration mg/m³ (ppm)							
	0-210 (0-50)	210-420 (50-100)	420-630 (100-150)	630-840 (150-200)	840-1050 (200-250)	1050-1260 (250-300)	> 1260 (> 300)	Totals
Plant Type								
Boat plants	127	144	80	32	25	23	48	479
Non-boat plants	26	47	29	5	3	1	2	113
Totals	153	191	109	37	28	24	50	592
Job Category								
Laminators	61	70	61	27	21	11	22	273
Chopper gun operators	37	62	40	25	13	17	30	224
Gel coaters	12	6	4	3			2	27
Totals	110	138	105	55	34	28	54	524
Plant Type (Exposure of Laminators)								
Boat plants	52	48	42	26	18	13	20	219
Non-boat plants	6	24	17	2	3	1	1	54
Totals	58	72	59	28	21	14	21	273

[a] Modified from: Schumacher et al. (1981).

Table 8. Styrene concentration during various processes in boat production

Process	Case	Personal sampling			Stationary sampling			
	N[a]	GM[b]	GSD[c]	Range[d]	N[e]	GM[b]	GSD[c]	Range[d]
Lamination over boat shell mould	A	25	500 (119)	6.8 (1.6)	143-1075 (34-256)	17	550 (131)	5.5 (1.3)	307-731 (73-174)
	B	9	273 (65)	5.8 (1.2)	193-378 (46-90)	15	281 (67)	5.9 (1.4)	160-546 (38-130)
Installation of ribs	laminators	3	71.4 (17)	7.1 (1.7)	42-118 (10-28)	20	33.6 (8)	10 (2.4)	8-214 (2-51)
	helpers	5	< 4.2 (< 1)	23.5 (5.6)	8 (D[f]-2)				
Installation of division plates	laminators	5	92.4 (22)	8.8 (2.1)	25-185 (6-44)	6	29.4 (7)	12.2 (2.9)	8-92 (2-22)
	helpers	5	D	-	(D-D)				
Auxiliary lamination on deck		5	54.6 (13)	16.8 (4)	8-181 (2-43)	5	96.6 (23)	7.1 (1.7)	59-164 (14-39)
Lamination on hold walls	A	4	128	1.4	(104-211)	3	269 (64)	19.3 (4.6)	55-1172 (13-279)
	B	21	127	1.3	(87-215)	20	269 (64)	7.6 (1.8)	101-1088 (24-259)
Equipment		8	2	2.8	(1- 17)	15	< 4.2 (< 1)	64.7 (15.4)	N[g]-38 (N[g]-9)
Plant floor	A					39	6	24.8 (5.9)	8-76 (2-18)
in general	B					14	< 1	69.7 (16.6)	N[a]-38 (N[a]-9)

[a] The number of workers equipped with personal samplers for 4 h work (1300-1700).
[b] The geometric mean.
[c] The geometric standard deviation.
[d] The minimum and maximum concentration observed.
[e] The number of sites monitored by stationary samplers for 4 h (1300-1700).
[f] Detected but not measurable (less than 4.2 mg/m³ (1 ppm).
[g] Not detected.

5. CHEMOBIOKINETICS AND BIOTRANSFORMATION

5.1 Uptake

5.1.1 Human studies

5.1.1.1 Uptake by inhalation

Pulmonary uptake of styrene concentrations of 67-164 mg/m³ (16-39 ppm), after a single breath or during exposures of up to 8 h, has been studied in human volunteers. Depending on exposure conditions, uptake varied from 45 to 66% (Bardodej et al., 1961; Fišerova-Bergerova & Teisinger, 1965; Bardodej & Bardodejova, 1970).

Åstrand et al. (1974) examined the pulmonary uptake of inhaled styrene (210 and 630 mg/m³; 50 and 150 ppm) in 14 healthy subjects at rest and during exercise over 30-min periods. Blood concentrations of styrene were measurable shortly after the onset of exposure indicating a rapid uptake. With light work (50 W exercise), the alveolar ventilation increased 3-fold, while the alveolar concentration of styrene barely changed. The concentration of styrene in arterial blood tripled.

Fernandez & Caperos (1977) measured the uptake of styrene in 6 volunteers at exposure levels ranging from 294 to 840 mg/m³ (70 to 200 ppm) for periods ranging from 4 to 8 h. The retention of styrene, measured from alveolar ventilation, varied from 82 to 93%.

In studies by Ramsey & Young (1978) and Ramsey et al. (1980) on 4 human volunteers, a single exposure to a styrene concentration of 336 mg/m³ (80 ppm) for 6 h resulted in a blood concentration at the end of exposure of about 800 µg/litre. A steady state concentration of about 900 µg/litre was predicted for repeated daily exposure to the same atmospheric concentrations. The authors assumed a 2-compartment model that allowed a zeroth order uptake rate of 99.6 mg/h to be calculated.

5.1.1.2 Uptake from the gastrointestinal tract

No studies have been found in the literature related to the uptake of styrene from the human gastrointestinal tract.

5.1.1.3 Uptake through the skin

Styrene is absorbed through human skin when applied in the form of a liquid, an aqueous solution, or a vapour. Rates of uptake of between 9 and 15 mg/cm² per h were reported when

liquid styrene was applied to the skin of the hands and forearm (Dutkiewicz & Tyras, 1967; 1968). Rates of uptake of 0.004-0.180 mg/cm^2 per h were reported when aqueous solutions containing mean styrene concentrations of between 66.5 and 269 mg/litre were applied. Skin absorption of styrene was some 30 times greater than that of aniline, benzene, or nitrobenzene.

Riihimäki & Pfäffli (1978) measured the percutaneous uptake of styrene vapour in 2 human volunteers, wearing thin cloth pyjamas and socks, who were exposed to a styrene vapour concentration of 2520 mg/m^3 (600 ppm) for 3.5 h, including 3 work intervals of 10 min each. Full-face respirators were used to prevent inhalation uptake and the temperature was maintained at 25°C with 50% relative humidity. Blood levels reached a peak 3 h after the start of exposure and then declined over the next 4 h. These results were equivalent to a pulmonary exposure of about 84 mg/m^3 (20 ppm) for an equal period of time.

5.1.2 Experimental animal studies

5.1.2.1 Uptake by inhalation

A recent review of studies of the uptake of styrene in several animal species indicated that styrene is distributed rapidly throughout the body, is stored in lipid-rich tissues, and is extensively metabolized (Santodonato et al., 1980). In a study on rats exposed to styrene concentrations in air of 336, 840, 2520, and 5040 mg/m^3 (80, 200, 600, and 1200 ppm) for up to 24 h, kinetic data obtained by Ramsey & Young (1978) obeyed a 2-compartment model. From the kinetic coefficients derived from the data, the authors predicted that there was little or no potential for bioaccumulation with repeated 8 h per day exposure to 336 mg/m^3 (80 ppm). They claimed that 95% of the predicted maximum blood concentration of styrene would be reached during the first day of exposure.

In another study in which rats with an indwelling jugular cannula were exposed to styrene atmospheres (Withey, 1978; Withey & Collins, 1979), a 2-compartment model was observed at exposure concentrations ranging from 210 to 8400 mg/m^3 (50 to 2000 ppm) for a period of 5 h. From the assumed zeroth-order kinetics for uptake together with the rate coefficients for distribution and elimination, it was possible to predict that the time to equilibrium or steady-state would be longer at higher exposure levels.

An autoradiographic study on the uptake of ^{14}C-styrene-8 and ^{14}C-styrene-ring by mice was carried out by Bergman (1979). Each animal inhaled the vapours from 10 µl of styrene in a small inhalation apparatus for 10 min. Extensive

uptake was observed in all organs and the potential for enterohepatic recycling was demonstrated by the appearance of the radiolabel in the bile. Styrene uptake in the mice was reported to be low compared with that in human subjects (less than one half) and this was attributed to the reduced respiratory frequency in the mouse, induced by the irritant action of the styrene vapour on the respiratory tract.

5.1.2.2 Uptake from the gastrointestinal tract

There have been few reports on the kinetics and the extent of uptake of styrene from the gastrointestinal tract in animals.

A study by Sauerhoff et al. (1976) on male and female rats, administered a single oral dose of ^{14}C-labelled styrene at 500 or 50 mg/kg body weight, showed that a greater fraction of unchanged styrene was excreted via the lung after administration of the higher dose, a result suggesting that saturable metabolic pathways were involved. The authors found that the uptake of styrene monomer from oral doses was greater for the female than for the male rats.

In a report of a preliminary investigation on the uptake kinetics of styrene from the gastrointestinal tract of rats (Withey, 1976), it was noted that the uptake and kinetic profile after a single dose, administered as an aqueous solution, was so rapid (peak values being obtained less than 4 min after the administration of the dose) that it was almost indistinguishable from the kinetics of an intravenous bolus dose. In contrast, when a similar dose was administered as a solution in vegetable oil, peak values were not obtained until 4 h after dosing.

5.1.2.3 Uptake through the skin

Wolf et al. (1956) applied unspecified amounts of liquid styrene to the ear or to the shaved abdomen of rabbits during periods of 2-4 weeks (10-20 applications). Moderate irritation and slight necrosis were observed. The authors claimed that, at the doses applied, there was no evidence that styrene was absorbed through the skin in amounts sufficient to cause acute, systemic toxicity.

The percutaneous uptake of styrene in rats has been measured by immersing the tail of the animal in liquid styrene for 1 h (Shugaev, 1969). Inhalation exposure was carefully avoided. Significant uptake of styrene by the liver and brain was estimated to be between 50 and 70 % of the concentrations found in the same organs after a 4-h inhalation exposure to a vapour concentration of 11.8 gm/m^3.

5.2 Distribution and Storage

5.2.1 Human studies

5.2.1.1 Controlled human studies

Studies on experimental animals have demonstrated that styrene is widely distributed throughout the tissues and organs and that it may accumulate in adipose tissue. Studies on the distribution of styrene in human volunteers have, necessarily, been limited to its quantitative analysis in blood, expired air, and adipose tissue.

In a study by Engström (1978), 7 male subjects were exposed to a styrene concentration of 210 mg/m^3 (50 ppm) during 30 min at rest and three 30-min periods on a bicycle ergometer set at a work intensity of 50, 100, or 150 W. The mean uptake of styrene was 490 mg. Specimens of subcutaneous adipose tissue were taken before and after exposure and at 0.5, 2, 4, and 21 h after exposure. Mean concentrations in the adipose tissue, up to 21 h after exposure, were of the same magnitude (3.6 mg/kg). From the concentration of styrene still detectable 13 days after exposure, it was possible to estimate an elimination half-time of between 2.2 and 4.0 days.

Ramsey & Young (1978) exposed volunteers to a styrene concentration of 336 mg/m^3 (80 ppm) for 6 h and observed that the decay of styrene concentration in the blood following the exposure fitted a 2-compartment model. They speculated that accumulation of styrene would not occur following repeated exposure to concentrations up to 840 mg/m^3 (200 ppm).

5.2.1.2 Occupational exposure studies

There have been a number of studies involving the determination of styrene concentrations in the blood and adipose tissue of workers occupationally exposed to styrene monomer. These studies were not only intended to investigate the correlation between levels in tissues and previous exposure to styrene but also to see if there was a potential for its accumulation and storage in adipose tissue. Wolff et al. (1977) studied 25 workers who allowed from 3 to 66 mg of subcutaneous gluteal adipose tissue to be taken. It was demonstrated that styrene persisted in this tissue for as long as 3 days following exposure, whereas urine metabolites and styrene in expired air persisted for up to 16 h after exposure. In an extension of this study (Wolff et al., 1978a), data on levels of styrene in the gluteal adipose tissue of an additional 25 workers suggested a correlation with blood styrene and urinary metabolite levels.

Engström et al. (1978a,b) measured air concentrations of styrene in a polymerization plant and obtained subcutaneous adipose samples from 3 workers. The TWA air concentrations ranged from 32 to 84 mg/m³ (7.6 to 20.2 ppm) and the mean daily styrene uptake of the 3 volunteers was 193, 343, and 558 mg, respectively. Adipose tissue concentrations were 2.8, 4.0, and 8.1 mg/kg, respectively, at the beginning of the working week, and 4.7, 7.7, and 11.6 mg/kg, at the end.

5.2.1.3 General population studies

Reports on the uptake of styrene in the general population are lacking.

5.2.2 Experimental animal studies

There have been a number of reports on the distribution of controlled doses of styrene monomer administered by different routes in several animal species.

In studies on mice and rats, Shugaev (1969) showed that, after inhalation exposure to the median lethal concentration (LC_{50}), for 2 and 4 h, respectively, there was a positive correlation between the brain concentration of styrene and lethality. Styrene levels in the perirenal adipose tissue were some 5 times higher than those in the brain, liver, kidney, and spleen.

Savolainen & Vainio (1977) studied the organ distribution of radiolabelled styrene and styrene epoxide following an intraperitoneal injection of 577 µmol, 3, 6, and 24 h after dosing. Three hours after dosing, the highest concentration of styrene was found in the kidneys followed by the brain, duodenum, liver, spinal cord, lungs, and blood. The kidney concentration was 19 times higher than that in the blood. After 6 h, levels in the duodenum and brain had decreased whereas levels in the blood had increased.

The distribution of ^{14}C-styrene-8 and ^{14}C-styrene-ring was studied in male mice after a 10-min inhalation exposure (Bergman, 1979). Animals were killed at 0.5, 1, 2, 4, 8, 24, and 48 h after exposure and subjected to whole-body autoradiography. The autoradiograms obtained shortly after exposure showed that the radioactivity was localized in the bronchi, lungs, and liver, for both styrene labelled in the side chain and the ring-labelled compound. Styrene was apparently cleared from the nervous tissues in less than 1 h, since no radiolabelled material was detected at 0.5 h. However, it was retained in adipose tissues for up to 48 h after dosing. Again, a large proportion of the radioactivity was found in the bile and kidney suggesting that these routes are the primary routes of excretion. Radiolabelled material

appeared in the intestinal contents within 1 h of exposure and persisted for at least 24 h.

A recent inhalation study on rats (Carlsson, 1981) showed that the largest amounts of ^{14}C-styrene and its metabolites were found in the kidneys, after an 8-h exposure to 184 mg/m^3 (45 ppm). The styrene concentrations were also high in subcutaneous adipose tissue; the concentrations in the cerebrum, cerebellum, and muscles amounted to about 70% of the styrene concentration in arterial blood.

The distribution of styrene was examined in male Wistar rats after intravenous administration at 4.01 or 13.37 mg/kg body weight or after a 5 h inhalation exposure at 6 concentrations between 227 and 9408 mg/m^3 (54 and 2240 ppm) (Withey, 1976; Withey & Collins, 1977, 1979). Animals were killed immediately after the termination of the vapour exposure, and the blood, heart, kidney, liver, spleen, brain, and perirenal fat were analysed for their styrene content. At all exposure levels except the lowest, styrene concentrations in all organs were higher than that in the blood. At the lowest level of exposure, the concentration in the kidney was at least 10 times greater than that in any other organ except the perirenal fat. At higher levels of exposure, the highest concentration was found in the liver. Levels of styrene in organs, 8 min after intravenous administration of 13.37 mg/kg were found in the following descending order: liver > kidney > brain > blood > heart and spleen. For the lower dose (4.01 mg/kg), the levels in these organs were of the same order of distribution, but lower than that in the blood, suggesting that, as in the case of the inhalation study, the distribution to the major organs varied with dose.

5.3 Biotransformation

The first step in the metabolic transformation of styrene is its oxidation, catalysed by cytochrome P-450 dependent monooxygenases, to oxirane derivatives in the aliphatic chain or in the aromatic ring (Fig. 1).

The oxidation of styrene to styrene 7,8-oxide has been demonstrated *in vitro* using rat or rabbit liver microsomes (Leibman & Ortiz, 1969; Leibman, 1975; Belvedere et al., 1977; Duverger-van Bogaert et al., 1978; Watabe et al., 1978), rat liver nuclei (Gazzotti et al., 1980) or rat, rabbit, or guinea-pig extrahepatic microsomes (Cantoni et al., 1978). The major route of styrene metabolism is believed to proceed via this epoxide intermediate.

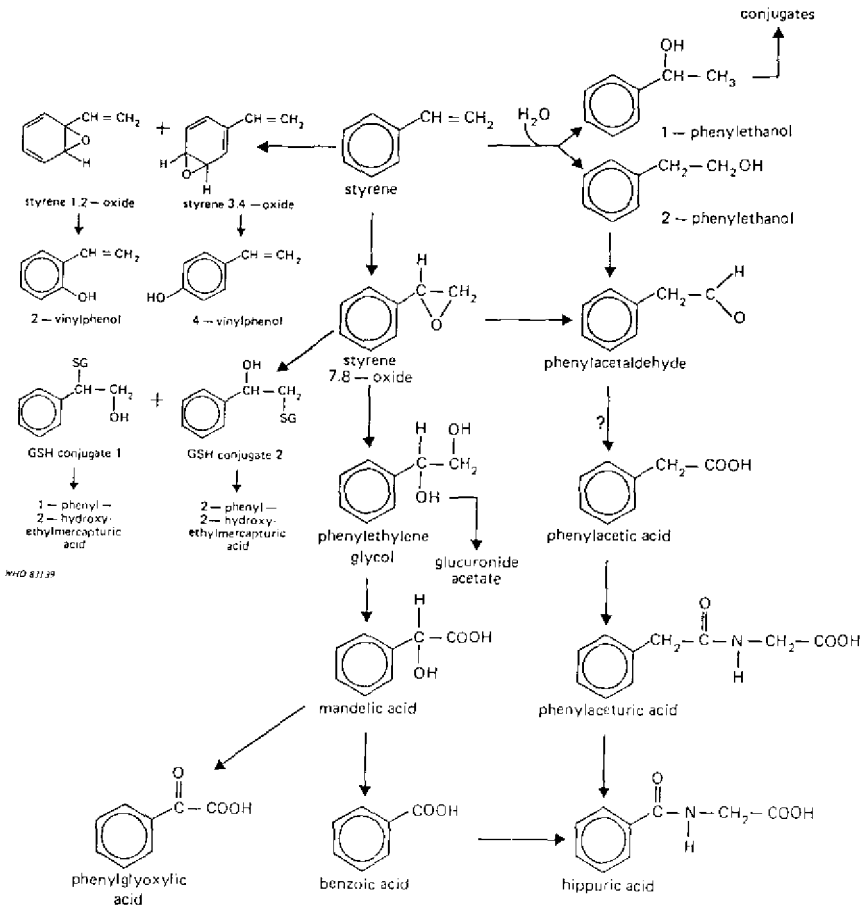

Fig. 1. Main metabolic pathways for styrene. Adapted from: Ohtsuji & Ikeda (1971); Leibman (1975); Bardoděj (1978); Vainio (1978); Watabe et al. (1978); Hiratsuka (1982a).

Styrene has been shown to be epoxidized to (R)- and (S)-styrene 7,8-oxides in the ratio 1:1.3 by rat liver microsomal cytochrome P-450 (Watabe et al., 1981a). The (R)- and (S)-oxides are hydrolysed in a regiospecific manner by rat liver microsomal epoxide hydrolase to (R)- and (S)-phenylethylene glycols in the ratio of 1:4 (Watabe et al., 1981a). Styrene oxides are also conjugated with glutathione (Oesch, 1973; James et al., 1975; Pacheka et al., 1979). Rat liver cytosolic glutathione transferases convert the (R)- and (S)-oxides stereoselectively to S-(1-phenyl-2-hydroxyethyl)-glutathione (GSH conjugate 1) and S-(2-phenyl-2-hydroxyethyl)-glutathione (GSH conjugate 2), respectively (Watabe et al., 1981b). Conjugates 1 and 2 would be directly correlated with the urinary N-acetyl-S-cysteine conjugates, namely 1-phenyl-2-hydroxyethyl and 2-phenyl-2-hydroxyethylmercapturic acids, respectively (James & White, 1967; Seutter-Berlage et al. 1978; Watabe et al., 1982c).

Rats administered styrene intraperitoneally (i.p.) also excreted 2-vinylphenol and 4-vinylphenol in conjugated forms in the urine (Hiratsuka et al., 1982a). Styrene 1,2- and 3,4-oxides administered i.p. to rats have been demonstrated in the urine as 2-vinylphenol and 4-vinylphenol, respectively (Hiratsuka et al., 1982a). However, 3-vinylphenol has not been detected in the urine (Bakke & Scheline, 1970; Pantarotto et al., 1978; Watabe et al., 1978). Other minor metabolites are 1- and 2-phenylethanol (Delbressine et al., 1980).

Comparing the kinetic parameters of styrene monooxygenase and styrene 7,8-oxide hydrolase in rat liver microsomes (Cantoni et al., 1978; Duverger-van Bogaert et al., 1978), it appears that the conversion of styrene 7,8-oxide into styrene glycol is preferred. The ratio between styrene epoxide hydrolase and monooxygenase in rat liver was 2-3 times higher than those found in mouse and rabbit liver (Cantoni et al., 1978).

Styrene glycol is partly transformed into a glucuronide that has been detected in the urine of styrene-treated rabbits (El Masri et al., 1958). The major metabolic pathway involves the sequential oxidation to mandelic, phenylglyoxylic, and benzoic acids, which have been identified in urine (Bardoděj, 1964; James & White, 1967; Ohtsuji & Ikeda, 1971). Quantitative differences have been observed between various species, hippuric acid being quantitatively the most relevant urinary metabolite in the rabbit (El Masri et al., 1958), phenylglyoxylic acid in the rat (Bardoděj et al., 1971; Watanabe et al., 1982c) and mandelic acid in man (Bardoděj, 1964; Bardoděj & Bardodějova, 1970). Minor metabolites identified include parahydroxymandelic, parahydroxyphenylglyoxylic, and parahydroxybenzoic acids (Bakke & Sheline, 1970; Pantarotto et al., 1978).

The metabolism of styrene is enhanced by phenobarbital pretreatment and is inhibited by SKF 525-A, an inhibitor of monooxygenases (Ohtsuji & Ikeda, 1971).

Styrene 7,8-oxide either binds covalently to macromolecules such as microsomal proteins (Marniemi et al., 1977; Watabe et al., 1978) or conjugates with glutathione non-enzymatically and in the presence of a transferase (Oesch, 1973; James et al., 1975; Pachecka et al., 1979). Phenobarbital pretreatment of the rats enhances, whereas carbon tetrachloride administration reduces, the NADPH dependent-binding to microsomal protein in vitro (Watabe et al., 1978).

Prolonged exposure to a styrene vapour concentration of 1260 mg/m^3 (300 ppm) leads to an enhancement of the metabolic elimination of the compound (Vainio et al., 1979) resulting in decreasing body burden during continued exposure.

Though numerous metabolic pathways have been found in animal studies (mainly in the rat and rabbit), only a few have been identified in human beings. Analysis of the urine of human subjects exposed to styrene has revealed mandelic and phenylglyoxylic acids as the major metabolites indicating that, at least qualitatively, styrene metabolism in human beings follows pathways similar to those previously described in animal studies. The results of a study by Pfäffli et al. (1981) suggest that 4-vinylphenol is also excreted in the urine of workers exposed to styrene. Though this metabolite represented only 0.3 % of the urinary concentration of mandelic acid, there was a good correlation between the excretion of the two metabolites.

Recent data indicate that human whole blood lymphocyte cultures apparently catalyse the oxidation of styrene to styrene 7,8-oxide (Norppa et al., 1980a). Belvedere & Tursi (1981) have shown that both human lymphocytes and erythrocytes are capable of converting styrene to styrene 7,8-oxide. In whole blood, red blood cells may be more important in this conversion, because they are present in greater numbers than lymphocytes.

5.4 Elimination

5.4.1 Human studies

5.4.1.1 Controlled human studies

In an inhalation study by Fišerova-Bergerova & Teisinger (1965), 7 human volunteers were exposed to styrene levels of 63-160 mg/m^3 (15-38 ppm) for 5 h. From analysis of the alveolar air, the authors found that pulmonary elimination followed a bi-exponential curve indicating a 2-compartment model.

Stewart et al. (1968) found that styrene elimination from the lungs, after inhalation exposure, was rapid and bi-exponential but that the rates of elimination varied with the level and duration of exposure. It was apparent that subjects who were exposed to a styrene concentration of 1579 mg/m³ (376 ppm) for 1 h had lower elimination rate coefficients for the terminal phase than those exposed to 907 mg/m³ (216 ppm) and 214 mg/m³ (51 ppm) for 1 h. No values for the rate coefficients were given in this report but it was calculated that one subject, who had been exposed to 491 mg/m³ (117 ppm) for 2 h, exhaled 1.2% of the total amount of styrene absorbed during the first 4 h after exposure. Exhaled styrene was detected up to 24 h after the termination of a 30-min exposure to concentrations of 210 or 630 mg/m³ (50 or 150 ppm) (Åstrand et al., 1974).

The exhalation of styrene after pulmonary exposure was also examined in human volunteers by Fernandéz & Caperos (1977). Six subjects were exposed to vapour concentrations of 294-840 mg/m³ (70-200 ppm) for periods of 4 or 8 h. The concentration of styrene in the expired alveolar air was reported to decline rapidly during the first hour following termination of exposure, at all exposure concentrations, and 2.6% of the total absorbed dose was considered to be eliminated via the lung.

Exhalation of styrene after inhalation exposure to a styrene concentration of 210 mg/m³ (50 ppm) was measured in 7 volunteers for 4 consecutive 30-min periods either resting or in a work cycle (Engström et al., 1978b). The exhalation was followed for up to 19 h after exposure by monitoring styrene concentrations in the expired air; some 31% of the retained styrene was estimated to be eliminated via the lungs. In the same study, styrene eliminated from adipose tissue was reported to have a half-life of from 2 to 4 days, which led the authors to conclude that repeated daily exposure to styrene could lead to bioaccumulation.

In a study by Ramsey & Young (1978), 4 male human volunteers were exposed to a styrene concentration of 336 mg/m³ (80 ppm) for 6 h and the styrene in the blood and expired air monitored for 41 h after exposure. The styrene concentration in blood was found to decay in a bi-exponential fashion, typical of a linear 2-compartment model. The rapid initial elimination phase yielded a half-time of 0.58 h and, for the slower terminal phase, a value of 13.0 h was reported. The concentration of styrene in the expired air for the same volunteers showed a biphasic log-linear decline similar to that observed for the blood concentrations.

In a study designed to investigate the skin absorption of styrene (Riihimäki & Pfäffli, 1978), two subjects wearing air-supplied respirators were exposed to a styrene concentration

of 2520 mg/m³ (600 ppm) for 3.5 h while performing light work. Bi-exponential elimination data were obtained both from analysis of venous blood and of alveolar air and the authors reported a value of about 1 h for the half-time of the rapid phase and about 10 h for the slower phase.

Studies conducted on 6 male volunteers at styrene exposures varying from 865 mg/m³ (206 ppm) for 8 h to 294 mg/m³ (70 ppm) for 4 h gave alveolar concentration decay curves that the authors claimed were best described by a 3-compartment model (Fernandez & Caperos, 1977). The rate coefficients for the three phases had half-lives that ranged from 0.06 to 0.61 h for the rapid phase, 1.01 to 3.75 h for the intermediate phase, and 10.05 to 24.75 h for the slowest phase.

A study on the rate of excretion of the urinary metabolites of styrene, phenylglyoxylic and mandelic acids (Guillemin & Bauer, 1978) was carried out on 9 human subjects after pulmonary exposure to styrene at 168 or 840 mg/m³ (40 or 200 ppm) for 4-8 h. The authors found that the rate coefficients for the urinary excretion of metabolites were unaffected by the duration or level of exposure and claimed that if the amounts of both metabolites were totalled over 4 days following exposure, reliable measurements of exposure could be obtained. For mandelic acid, the first and second phase half-times were evaluated to be respectively 3.9 h and 24.7 h, while for phenolylyoxylic acid the half-time was calculated to be 10.5 h (Guillemin & Bauer, 1979).

5.4.1.2 Occupational exposure studies

Of the numerous studies that have been conducted on styrene-exposed workers (Härkönen, 1978), only a few have been devoted to the assessment of metabolite production.

In a study by Götell et al. (1972), a group of 17 workers was divided into 3 exposure subgroups, the highest at 987-1226 mg/m³ (235-292 ppm), an intermediate group at 374-584 mg/m³ (89-139 ppm), and the lowest group at 71-134 mg/m³ (17-32 ppm). Decay curves of styrene in expired air for the 3 groups had a multi-exponential form but no kinetic parameters were cited. It was evident, however, that the terminal elimination phase was markedly faster for the group receiving the highest exposure.

The rate of appearance of mandelic acid in the urine has been shown to be biphasic, half times being 9 h and 16 h, respectively (Engström, K., et al., 1976). In a preliminary report on workers, with limited time points, monophasic elimination with half-times of 8.5 and 7.8 h, respectively for phenylgly- oxylic and mandelic acid, was found (Ikeda &

Imamura, 1974). Considerable inter-subject variability has been reported by several authors (Philippe et al., 1971; Gotell et al., 1972; Härkönen et al., 1974; Gompertz, 1978).

On the basis of findings in animal studies, the excretion of hippuric acid was investigated in styrene-exposed workers. An increase in hippuric acid excretion was noted in styrene-exposed workers (Ikeda et al., 1974); similar levels and important fluctuations have been found in unexposed controls (Engström, K., et al., 1978).

5.4.1.3 General population studies

There have not been any reported studies on the elimination of styrene in the general population.

5.4.2 Experimental animal studies

Results of studies on rats subcutaneously injected with 500 mg/kg ^{14}C-labelled styrene showed that there was rapid distribution to tissues and organs within 1 h and that 85% of the radioactivity was eliminated within 24 h; 71% of the radioactivity appeared in the urine, 12% in expired air as carbon dioxide, 3% in the faeces, and about 3% as unchanged styrene in expired air (Danishefsky & Willhite, 1954).

A later study (Sauerhoff et al., 1976) demonstrated a similar rapid clearance of ring-labelled ^{14}C-styrene in male and female rats after oral administration of 50 or 500 mg/kg body weight. More than 90% of the radioactivity appeared in the urine within 72 h and no residual styrene was found in the tissues examined at that time. The urinary excretion of radioactivity followed a 2-compartment model at the low dose level with half-times of 1.29 and 8.13 h for the initial (alpha) and terminal (beta) phases, respectively. At the high dose, a mono-exponential urinary excretion was observed with a half-time of 6.74 h. About twice as much styrene was eliminated by the pulmonary route in male compared with female rats.

Biphasic excretion of radioactivity in the urine of rats was observed after inhalation of ^{14}C-styrene (252 or 2520 mg/m^3; 60 or 600 ppm) for 6 h with half-time values of 14.9 h for the lower and 19.2 h for the higher exposure concentration (Sauerhoff & Braun, 1976).

Inhalation exposure of rats to styrene concentrations varying from 336 to 5040 mg/m^3 (80 to 1200 ppm) for 6 h (Ramsey & Young, 1978) also showed evidence of dose-dependent kinetics. For the 15-fold increase in exposure level, the area generated under the blood level-time curve increased by a factor of 112. The elimination kinetics in the post-exposure period appeared to change from a bi-exponential curve to a

log-linear form with increasing exposure concentrations. At the 336 mg/m³ (80 ppm) dose level, the hybrid elimination rate coefficients for the initial and terminal phases were 0.26 h and 3.60 h, respectively.

Withey (1978) and Withey & Collins (1979) described the elimination kinetics of styrene in rats after 5 h inhalation exposure at levels that varied from 188 to 10 151 mg/m³ (44.8 to 2417 ppm). In contrast to the study by Ramsey & Young (1978), these authors reported a bi-exponential curve of elimination for all levels of exposure. The reported half-times for the initial and terminal phases ranged from 3.4 to 6.3 min and about 50 to 350 min, respectively.

Teramoto et al. (1978) examined the elimination rates of styrene from samples of the liver, brain, kidney, blood, spleen, muscle, and adipose tissue in rats after an inhalation exposure to 2940 mg/m³ (700 ppm) for 4 h. They found that all tissues had a similar elimination half-time of about 2 h with the exception of adipose tissue for which the styrene half-time was about 6 h.

To examine the accumulation of styrene in tissues, Savolainen & Pfäffli (1978) exposed rats to 1260 mg/m³ (300 ppm), for 6 h per day, and 5 days per week, for up to 11 weeks and then monitored the styrene concentrations in adipose tissue. Concentrations increased almost linearly, during the first 4 weeks of exposure and then decreased exponentially.

In the study reported by Bergman (1979) (section 5.2.2), mice were exposed through inhalation to 10 µl of volatilized radiolabelled styrene over a 10-min period. The amount of unchanged styrene exhaled over an 8-h period following exposure amounted to 2.9% of the calculated dose. The kinetics of elimination of radioactivity in exhaled air gave a biphasic curve with half-times of 15 and 275 min for the initial and terminal phases, respectively. Eight hours after dosing, high levels of radioactivity were found in the liver, kidney, lung, and adipose tissue.

Results of a study by Withey & Collins (1977) showed that the elimination of styrene after intravenous administration in rats also obeyed a 2-compartment model. Approximate rates of elimination from some individual organs were obtained for dose levels of approximately 1.3 and 4 mg/kg body weight. At the higher dose, the kinetics of styrene disappearance from blood, heart, brain, liver, spleen, and kidney appeared to follow a 1-compartment model with an elimination half-time of about 14.4 h in the same organs. At the lower dose, a biphasic elimination was observed with half-times of about 4.78 and 26 h for the rapid and slow phases, respectively.

5.5 Biological Monitoring of Styrene Uptake

The measurement of styrene in blood, subcutaneous fat, as well as in exhaled air has been suggested for the biological monitoring of styrene uptake. Because of the efficient metabolism of styrene and rapid excretion of the metabolites, mandelic and phenylglyoxylic acid are present in the urine in easily measurable amounts. The evaluation of the absorption level has usually been based either on the concentration of one of these metabolites (Ohtsuji & Ikeda, 1970; Burkiewicz et al., 1974; Härkönen et al., 1974; Vivoli & Vechhi, 1974; Engström, K., et al., 1976, 1978; Fernandez & Caperos, 1977; Caperos et al., 1979; Elia et al., 1980) or on their sum (Bardodej & Bardodějova, 1970; Philippe et al., 1971; Guillemin & Bauer, 1978, 1979; Wolff et al., 1978a, b; Elia et al., 1980). "End of exposure" spot specimens have usually been sampled and results corrected for the dilution of urine. Only this type of sample is dealt with in the following discussions.

A number of authors have attempted to correlate levels of styrene in air with levels of mandelic acid in urine, either from experimental data or field studies, and calculations of mandelic acid levels corresponding to styrene levels in air of 210, 420, and 630 mg/m³ (50, 100, and 150 ppm) are presented in Table 9. The differences between the calculated values could be explained by such factors as: type of exposure, sampling strategy, individual variability, and differences in analytical methods.

The use of unspecific analytical methods influences the results, especially at low levels of styrene exposure, giving too high values for metabolite concentrations in the urine (Engström & Rantanen, 1974).

In the case of occupational exposure, a number of additional elements may have to be taken into account. For example, in work situations, exposure is usually repeated and it has been shown that about 30% of the maximal mandelic acid concentration of the previous day can still be found in urine samples taken before work on the following day (Engström, K., et al., 1976). In another study, the amount of mandelic acid excreted at the end of the working shift was shown to increase in the course of the working week (Fernandez & Caperos, 1977).

Increased pulmonary ventilation with increased physical work load results in a larger total absorption of styrene and thus increased excretion of metabolites in the urine (Åstrand et al., 1974; Droz & Fernandez, 1977).

Table 9. Mandelic acid levels* corresponding to various concentrations of styrene in air

Styrene mg/m³ in air (ppm)		210 (50)	420 (100)	630 (150)	Remarks	Reference
mandelic acid	(a)	6	12	18	mandelic acid expressed as mg/g creatinine; spectrophotometry	Spasovski (1982)
	(a)	-	7	-	mandelic acid expressed as mg/g creatinine; gas chromatography experimental study	Guillemin & Bonet (1979)
	(b)	8	14	20	Density 1.024; high performance liquid chromatography field study	Ikeda et al (1982)
	(b)	10	20	-	Denisty 1.024; polarography; experimental study	Bardoděj (1978)
	(b)	9	19	27	Density 1.024; gas chromatography; field study	Engström, K. et al. (1978)
	(b)	14	26	34	Density 1.024 spectrophotometry field study	Härkönen et al. (1974)
	(b)	7-9	12-13	15-19	Density 1.024; spectrophotometry; field study	Götell et al. (1972)
	(b)	6	14	22	Density 1.024 gas chromatography field study	Elia et al. (1980)
	(b)	3	11	19	Density 1.024; gas chromatography field study	Fields et al. (1979)

*(a) = mg mandelic acid/g creatinine.
(b) = mmol mandelic acid/litre urine.

Fluctuations in styrene concentrations in the workroom air are very common in occupational exposure and may also contribute to the differences observed in Table 9. A short-term high peak exposure occurring shortly before the sampling time may result in a high mandelic acid concentration in the urine, even though the time-weighted average concentration of styrene is low (Engström, K., 1983).

The ratio of mandelic to phenylglyoxylic acid excreted at the end of exposure varied from about 1 to 4 in different studies. The ratio appears to increase at higher exposure levels (Ohtsuji & Ikeda, 1970; Riihimäki & Pfäffli, 1978; Caperos et al., 1979; Elia et al., 1980). The mandelic acid/phenolglyoxylic acid ratios were higher in urine samples taken at the end of exposure than in samples taken later (Philippe et al., 1971; Guillemin & Bauer, 1979). In terms of biological monitoring, it is claimed that the mandelic acid/phenolglyoxylic acid ratio in the urine may be of help in interpreting the exposure pattern (Philippe et al., 1971; Guillemin & Bauer, 1979).

The various factors influencing the excretion of these metabolites are not well known. It may be that such a difficulty could be overcome by determining mandelic and phenylglyoxylic acids and combining the results. This may explain the good agreement obtained in the two studies in Table 10 based on the sum of the levels of the two acids corresponding to styrene concentrations in air of 210, 420, and 630 mg/m^3 (50, 100 and 150 ppm).

Table 10. Sum of mandelic acid and phenylglyoxylic acid levels (mmol/litre) corresponding to various concentrations of styrene in air

Styrene mg/m^3 in air (ppm)	210 (50)	420 (100)	630 (150)	Remarks	Reference
Mandelic acid + phenylglyoxylic acid	8	16	24	density 1.024; gas chromatography; field study	Elia et al. (1980)
	11	19	27	density 1.024 aigu performance liquid chromatography; field study	Ikeda et al. (1982)

The determination of minor metabolites (4-vinylphenol and hippuric acid) for the biological monitoring of styrene exposure has also been reported (Ikeda et al., 1974; Pfäffli et al., 1981).

The difficulties of the biological monitoring of styrene uptake using urine samples cannot be overcome by selecting another medium, as even less experience is available with other media. Styrene concentrations in blood decline rapidly during the first hour following exposure, which makes the results strongly dependent on sampling time.

Styrene concentrations in exhaled air are in equilibrium with blood concentrations, which means that styrene levels in exhaled air are also sensitive to the changes in concentration in ambient air. A single sample of exhaled air at the end of exhalation can be used only for the evaluation of immediate past exposure. It has been suggested that extrapolation of a whole day's exposure is possible using several samples taken after exposure, from which a "decay curve" can be established (Fernandez & Caperos, 1977; Fields & Horstman, 1979). This method is inconvenient for the routine monitoring of workers.

The estimation of styrene concentrations in subcutaneous fat has been proposed as a method of indicating styrene body burden, even a long time after exposure, when styrene and its metabolites can no longer be detected in other specimens (Wolff et al., 1978). However, much more information about the kinetics of styrene in fat is needed before the relevance of this method can be evaluated. For obvious practical reasons, this technique cannot be used routinely.

6. EFFECTS ON EXPERIMENTAL SYSTEMS

6.1 Haematopoietic and Immune Systems

Spencer et al. (1942) did not observe any differences in erythrocyte count, haemoglobin concentration, total leukocyte count, and differential count between 18 rats exposed repeatedly for 7 h a day for 6 months to a styrene concentration of about 5460 mg/m^3 (1300 ppm) and 18 control animals. The test animals received a total of 137-139 exposures. The same authors did not see any significant haematological changes in 8 rabbits and 4 monkeys similarly exposed.

Wolf (1956) gave styrene orally at 66.7-667 mg/kg body weight to female rats for 6 months, 5 days a week, but did not observe any effects on the haematopoietic system.

In a study by Quast et al. (1978), styrene was administered orally to beagle dogs at 200, 400, and 600 mg/kg body weight for up to 19 months. At the highest dose, Heinz bodies were regularly detected in the blood erythrocytes of the dogs. At 400 mg/kg body weight, Heinz bodies were still frequently seen, but at 200 mg/kg body weight they were observed only sporadically in female dogs. Heinz bodies disappeared after withdrawal of styrene. Decreases in packed cell volume, red blood cell count, haemoglobin levels, and the erythrocyte sedimentation rate were also observed.

When styrene was fed to rabbits at doses of up to 250 mg/kg body weight for up to 216 days, severe impairment of the immunological defence system was indicated with the blood complement titre reduced and leukocyte phagocytic activity depressed (Sinitskij, 1969).

6.2 Nervous System and Behaviour

The acute neurotoxic effect of styrene is depression of the central nervous system (Roth, 1979). However, the narcotic effect of styrene glycol is at least twice as potent as that of styrene (Parkki et al., 1976) and styrene 7,8-oxide is more toxic than both styrene and styrene glycol with a lethal dose 4-5 times lower than that of the parent compound (Ohtsuji & Ikeda, 1971). Central nervous system depression follows treatment with styrene 7,8-oxide. After a single intraperitoneal injection both styrene and styrene oxide have been shown to bind covalently to macromolecules in the central nervous system (Savolainen & Vainio, 1977). In a study on rats by Shugaev (1969), in which styrene was injected intraperitoneally, the maximum concentration of styrene in brain tissue was 250 mg/kg (range 180-324 mg/kg).

Toxic action in the central nervous system in prolonged (11 weeks) low-level inhalation exposure to a styrene concentration of 1260 mg/m³ (300 ppm) led to minor changes in axonal proteins (Savolainen & Pfäffli, 1977). Concomitant neurophysiological studies, however, failed to demonstrate any reduction in the peripheral motor conduction velocities (Seppäläinen, 1978), a suggested clinical feature of the toxicity of styrene in human subjects.

In studies by Zaprianov & Bainova (1979) on male rats, repeated oral doses of styrene (1250 mg/kg body weight for 7 days) decreased monoamine oxidase (EC 1.4.3.4) activity. The authors concluded that the inhibition of monoamine oxidase activity in total brain may be regarded as a possible link in the pathogenesis of reported occupational neurophysiological effects of styrene.

Polystyrene containers were toxic to nerve cells in a closed tissue culture system, possibly because of the release of unreacted styrene in the medium (Mithen et al., 1980).

In an open-field test, styrene vapour at 1260 mg/m³ (300 ppm) had marginal effects on animal behaviour, even when pronounced neurochemical effects were taken into account (Savolainen et al., 1980).

In a 6-month study, female rats exposed to 200 and 2000 mg/m³ of styrene exhibited enhanced spontaneous activity. Long-term memory was impaired in male rats after a 4-month exposure (Vergieva & Zajkov, 1981).

6.3 Kidneys and the Urinary Tract

Oral treatment of rats with styrene at 400 mg/kg body weight (administered in olive oil, once a day for 132 days during 6 months) caused an increase in kidney weight. A lower daily dose of 133 mg/kg body weight did not have any apparent effect (Wolff et al., 1956).

Vainio et al. (1979) reported that intermittent 11-week inhalation exposure of rats to low styrene vapour concentrations of 1260 mg/m³ (300 ppm), 6 h/day, 5 days a week, enhanced the activities of both drug conjugating (epoxide hydratase (EC 4.2.1.63), UDP glucuronosyl transferase (EC 2.4.1.17), and hydroxylating (ethoxycoumarin O-deethylase, cytochrome P-450) enzymes in the kidneys.

6.4 Gastrointestinal Tract

No information was available concerning the effect of styrene on the gastrointestinal tract.

6.5 Liver

Mild liver damage, particularly in the parenchymal cells, was reported after a single exposure of rats to styrene vapour at 10 500 mg/m^3 (2500 ppm) for up to 21 h (Spencer et al., 1942). The same authors also reported increases in liver weight in rats repeatedly exposed to styrene vapour at 5460 mg/m^3 (1300 ppm) for 130-139 days. However, they did not find any liver damage in guinea-pigs exposed for similar periods to a styrene concentration of 2730 mg/m^3 (650 ppm) or in rabbits and monkeys exposed to 5460 mg/m^3 (1300 ppm).

Intermittent 11-week inhalation exposure of rats to a styrene concentration of 1260 mg/m^3 (300 ppm) (6 h/day, 5 days/week) caused histological liver alterations consisting of hydropic degeneration, and steatosis of parenchymal cells and congestion. Enhanced activity of both the drug hydroxylating (ethoxycoumarin O-deethylase, cytochrome P-450) and conjugating (epoxide hydrolase (EC 3.3.2.3), UDP glucuronosyltransferase (EC 2.4.1.42)) enzymes in the liver was detected (Vainio et al., 1979). In a skin-painting study on rats, styrene at 4 and 8 ml/kg body weight induced dose-related fatty infiltration of liver cells that was reversible after 2 weeks without exposure (Burkova et al., 1982).

Liver cell necrosis was noted in a long-term carcinogenicity bioassay in several rats in a group exposed to a high dose of styrene (2000 mg/kg body weight by gavage, 5 days a week, for 78 weeks) (US National Cancer Institute, 1979).

The intermittent inhalation of styrene vapour at a concentration of 1260 mg/m^3 (300 ppm) in air decreased significantly the reduced glutathione (GSH) concentration in rat liver (Elovaara et al., 1979). Vainio & Mäkinen (1977) demonstrated a clear species difference in sensitivity to styrene-induced depletion of GSH content. The mouse was the most sensitive and the rat the most resistant species. The decrease in GSH in liver was dose-dependent. A slight decrease was observed in rats exposed to a styrene concentration in air of 420 mg/m^3 (100 ppm) (Vainio et al., 1979). When isolated hepatocytes were incubated in the presence of styrene, there was also a dose- and time-dependent decrease in GSH concentrations (Zitting et al., 1980).

When the hepatic GSH in Syrian hamsters decreased to less than 1 mmol/litre as a result of a high intragastric dose of styrene (4.5 g/kg body weight), serum aminotransferase activity increased (Parkki, 1978).

Quast et al. (1979) observed an increased amount of hemosiderin pigment in liver reticuloendothelial cells of male and female beagle dogs administered styrene orally at 400 or 600 mg/kg body weight, 7 days a week, for up to 19 months.

The higher dose also increased the number of hepatocellular intranuclear acidophilic crystalline inclusions.

6.6 Cardiovascular System

No information was available concerning possible effects of styrene on the cardiovascular system.

6.7 Respiratory System

A single exposure of rats to styrene at 5460-42 000 mg/m^3 (1300-10 000 ppm) for 1-4 h caused mucous membrane irritation as evidenced by lacrymation, nasal discharge, and salivation, accompanied by aggressive scratching and rubbing of the eyes and nose. The irritation was particularly severe at higher concentrations (Spencer et al., 1942). The degree of acute pulmonary damage varied, in general, with the vapour concentration of styrene and the length of exposure. When the exposure was long enough to cause death in some of the exposed animals, marked pulmonary lesions were induced at all concentrations studied. Pulmonary changes varied from slight congestion to congestion, multiple haemorrhage, exudation, and various degrees of leukocytic infiltration. When another group of animals (60 rats, 94 guinea-pigs, 12 rabbits, 4 monkeys) were exposed to a styrene concentration of 5460 mg/m^3 (1300 ppm) for 7-8 h/day, 5 days a week, over a period of at least 6 months, microscopic examination of various tissues, including the lung, did not reveal specific changes in any of the animals except the guinea-pigs. About 10% of all guinea-pigs that were exposed repeatedly to styrene at 5460 mg/m^3 (1300 ppm) died after several exposures; microscopic examination of the dead animals revealed pronounced lung irritation, which was characterized by general acute inflammatory response. Respiratory insufficiency was the cause of death (Spencer et al., 1942).

Wolf et al. (1956) exposed animals by inhalation to a styrene concentration of 5460 mg/m^3 (1300 ppm) for 7 h/day, for 214-360 days. They found slight eye and nasal irritation in rats and guinea-pigs, but no effects in rabbits and rhesus monkeys. However, when rats and guinea-pigs were exposed to a styrene concentration of 2730 mg/m^3 (650 ppm), no effects were observed.

According to Alarie (1973), the concentration of styrene in air that reduced the respiratory frequency in mice to 50% was 666 mg/m^3 (95% confidence limits 574-758 mg/m^3).

When rats were exposed to styrene at 1260 mg/m^3 (300 ppm) for 6 h daily, 5 days a week for 2-11 weeks, no significant increase in UDP glucuronosyltransferase activity was observed in the lungs but, after 2 and 4 weeks exposure,

the free glutathione content in the lung was lower ($P < 0.05$) than that in the controls (Vainio et al., 1979).

6.8 Endocrine Organs

The adverse effects of styrene on the endocrine organs have been insufficiently studied. While Izjumova (1972) reported that repeated exposure to styrene concentrations of 5-50 mg/m³ (1-12 ppm) for up to 4 months prolonged the estrus cycle in rats, the data were inconsistent from month to month, and appeared not to be dose-dependent.

6.9 Carcinogenic Effects

6.9.1 Styrene

6.9.1.1 Oral administration

(a) Mouse

In a carcinogenesis bioassay (US National Cancer Institute, 1979; Chu et al., 1981), 2 groups each of 50 male and 50 female B6C3F1 mice, 6 weeks of age, were given styrene at 150 and 300 mg/kg body weight (at least 0.3% impurities) in corn oil by gavage, 5 days per week, for 78 weeks, and then observed for an additional 13 weeks. A group of 20 males and 20 females receiving corn oil only (10 ml/kg body weight) served as vehicle controls. At the end of the study, 78%, 92%, and 100% of the male mice and 76%, 80%, and 80% of the females were alive in the high-dose, low-dose, and control groups, respectively. An increased incidence of adenomas and carcinomas of the lung was seen in treated male mice, i.e., 3 adenomas and 3 carcinomas in 44 animals in the low-dose group, and 4 adenomas and 5 carcinomas in 43 animals in the high-dose group, compared with none in the 20 controls. The increased incidence of both types of lung tumours combined was statistically significant ($P = 0.02$) in the high-dose group compared with the vehicle-treated controls and also in the Cochrane-Armitage test for linear trend ($P = 0.02$). It should be noted that the number of control animals was small (40) compared with the treated group (100), that the historical vehicle-treated controls were inadequate, and that the incidence of neoplasms in historical untreated controls was 12% (32/271). For these reasons, it was not possible for the Task Group to conclude that styrene was carcinogenic in this study, even though the results of the study were positive.

(b) Rat

In carcinogenic bioassays (US National Cancer Institute, 1979; Chu et al., 1981), groups of 50 male and 50 female

Fischer 344 rats, 6 weeks of age, were given styrene (at least 0.3% impurities) in corn oil, by gavage, 5 days per week, either at 1000 or 2000 mg/kg body weight for 78 weeks with further observation for 27 weeks, or at 500 mg/kg body weight for 103 weeks with further observation for 1 week. Two groups, each of 20 males and 20 females served as vehicle controls. A high mortality was seen in the high-dose group; only 12% of the males survived 53 weeks of treatment and 14% of the females survived 70 weeks of treatment. Hepatic necrosis was observed in several rats. At the 90th week of the study, 94%, 88%, 85%, and 90% of the males and 92%, 92%, 75%, and 90% of the females were alive in the medium-dose, low-dose, and the 2 control groups, respectively. There was no statistically significant difference between the groups in tumour incidence at any site (National Cancer Institute, 1979). The Task Group noted the high mortality in the high-dose group and the small numbers of animals in the control groups.

6.9.1.2 Inhalation exposure

Rat

In a study conducted by Jersey et al. (1978), 2 groups of 84 and 86 male, and 2 groups each of 85 female Sprague-Dawley rats of the Spartan substrain, 7-8 weeks of age, were exposed by inhalation to "production grade" styrene (minimum purity 99.5%) containing approximately 5 ppm t-butylcatechol. They were exposed 6 h/day, 5 days/week, for 18.3 months, if male, and for 20.7 months, if female. Exposure duration was based on the time when mortality reached 50% in one of the test groups of that sex. Initial concentrations of styrene in air were either 5040 mg/m^3 (1200 ppm) or 2520 mg/m^3 (600 ppm). The high level was reduced to 4200 mg/m^3 (1000 ppm) after 2 months because of excessive toxicity. Another group of 85 males and 85 females served as untreated controls. At the end of the 2-year study, 5, 18, and 6 males and 30, 30, and 22 females had survived in the control, low-, and high-dose groups, respectively. An excessive mortality from chronic pneumonia occurred in the control and high-dose male groups. An increased (but not statistically significant) combined incidence of leukaemias and lymphosarcomas was seen: 1/84 males and 6/85 females in the high-dose groups, 5/86 males and 6/85 females in the low-dose groups, 1/85 males and 1/85 females in the control groups. If the total incidence of leukaemias/lymphosarcomas in females is compared with historical controls, it becomes statistically significant ($P < 0.05$). A statistically significant increase in the incidence of mammary adenocarcinomas was seen in the low-dose

female group (7/85 versus 1/85 in controls), but the incidence was not statistically significant in comparison with historical controls. Though these data are inconclusive, they are interpreted by Jersey et al. (1978) as suggesting an association between the exposure of the female rats to styrene and an increased incidence of tumours of the leukaemia/lymphosarcoma types. The Task Group was unable to evaluate this study because of the high mortality.

6.9.1.3 Pre- and postnatal exposure

(a) Mouse

In the studies of Ponomarkov & Tomatis (1978), 29 pregnant 0_{20} mice were each given a single treatment of styrene at 1350 mg/kg body weight (99% pure dissolved in 0.1 ml olive oil) by stomach tube on the 17th day of gestation. A control group of 9 pregnant animals received olive oil alone. The neonatal mortality rate in the offspring of styrene-treated mice was 43%, compared with 22% in the controls. The same dose of styrene was then administered weekly, by stomach tube, to 45 male and 39 female progeny from weaning to 16 weeks of age, at which time the treatment was stopped because of the high rate of mortality (64% alive at 20 weeks). The study was terminated at 100 weeks, when all the animals had died. No differences in tumour incidence were found between mothers treated with styrene and those given olive oil. In the progeny that had received weekly treatments, lung tumours (adenomas and adenocarcinomas) were found in 20/23 males and 32/32 females, compared with 8/19 and 14/21 in olive oil-treated controls ($P < 0.01$, $P < 0.01$) and compared with 34/53 and 25/47 in untreated controls ($P < 0.05$, $P < 0.001$). No differences in tumour incidence at sites other than the lung were seen in the progeny, compared with olive oil-treated or untreated controls.

In the same studies (Ponomarkov & Tomatis, 1978), 15 pregnant C_{57} black mice were each given a single treatment of styrene at 300 mg/kg body weight dissolved in 0.1 ml olive oil, by stomach tube, on the 17th day of gestation. A control group of 5 pregnant animals received olive oil alone. The same amount of styrene was then given weekly by stomach tube to 27 male and 27 female progeny from weaning up to 120 weeks of age, at which time the survivors, 15 males and 12 females, were killed. Control progeny (12 males and 13 females) received olive oil for 120 weeks at which time 7 males and 4 females survived. In mothers treated with a single dose of styrene, lymphomas were observed in 10/12 animals, compared with 3/5 in the olive oil-treated controls. In treated male progeny, liver tumours (hepatocellular carcinomas) were found in 3/24 animals, compared with 1/12 olive oil-treated and 1/47

untreated controls (both hepatocellular adenomas). The increased incidence of these tumours in styrene-treated animals compared with controls was not statistically significant. The small number of animals was noted by the Task Group.

(b) Rat

Twenty-one female BDIV rats were each given a single treatment of styrene at 1350 mg/kg body weight dissolved in olive oil, by stomach tube, on the 17th day of gestation (Ponomarkov & Tomatis, 1978). A control group of 10 pregnant rats received 0.3 ml olive oil alone. The neonatal mortality rate in the offspring was 10% in treated rats and 2.5% in olive oil-treated controls. Subsequently, doses of 500 mg/kg body weight were administered weekly by stomach tube to 73 male and 71 female progeny from weaning up to 120 weeks of age, at which time the survivors, 8 males and 20 females, were killed. Controls (36 males and 39 females) received olive oil alone for 120 weeks. Two stomach tumours and 1 hepatocellular adenoma were seen in styrene-treated females and one stomach tumour in styrene-treated males. One stomach tumour was also found in olive-oil-treated female controls. The difference in the incidence of these and other tumours in styrene-treated rats compared with controls was not statistically significant.

6.9.2 Styrene 7,8-oxide

6.9.2.1 Dermal exposure

Mouse

Forty 13-week-old C3H mice were painted on the clipped dorsal skin with a 5% solution of styrene 7,8-oxide in acetone, 3 times weekly for life (Weil et al., 1963). No skin tumours were observed in 33 animals that survived for 17-24 months (37 mice were alive at 12 months). Another group of C3H mice were similarly painted with a 10% solution of styrene oxide in acetone; 18 mice survived 12 months, only 2 mice survived 17 months, and no skin tumours were observed .

In another study by Van Duuren et al. (1963), the clipped dorsal skin of 30, 8-week-old male Swiss ICR/Ha mice was treated 3 times weekly, throughout the life span, with 0.1 ml of a 10% solution of styrene oxide in benzene. Median survival time was 431 days in the styrene oxide-treated group and 262 and 412 days, respectively, in the groups of 30 and 60 benzene-treated controls. Three styrene-oxide-treated animals and 11 (out of 150) benzene controls developed skin tumours. This difference was not statistically significant. One of the tumours in each group was a squamous-cell carcinoma.

6.9.2.2 Oral administration

Rat

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks of age, were given either 50 or 250 mg/kg body weight, per day, of styrene oxide (purity unspecified) dissolved in olive oil, by stomach tube, 4 or 5 days per week, for 52 weeks (Maltoni et al., 1979). Forty male and 40 female controls received olive oil alone. The animals were allowed to live until natural death or were killed 156 weeks after the start of the study. Preliminary results included only stomach cancer incidence in animals dying within the first 135 weeks. The numbers of animals alive at the appearance of the first tumour (51 weeks) were: controls, 37 males and 28 females; low-dose, 31 males and 31 females; high-dose, 28 males and 30 females. The numbers of papillomas of the stomach were: 0/37 and 0/28 in controls; 0/31 and 2/31 in low-dose animals; and 3/28 and 6/30 in high-dose animals. The respective numbers for squamous-cell carcinomas in situ were: 0/37 and 0/28; 5/31 and 6/31; and 11/28 and 12/30. Invasive squamous-cell carcinomas occurred in 0/37 and 0/28; 2/31 and 1/31; and 4/2; and 6/30, respectively.

Fourteen female rats were given, by gavage, a single dose of styrene oxide at 200 mg/kg body weight in olive oil (0.3 ml) on day 17 of pregnancy (estimated total dose, 0.04 g). Their progeny (62 females and 43 males) received 96 weekly doses of 100-150 mg/kg body weight in olive oil (0.2 ml), from weaning (4 weeks of age) until termination of the study (120 weeks) (estimated total doses, 2.5 g for females and 5.0 g for males). Similar groups of controls (14 pregnant females, 55 female and 49 male progeny) were given only olive oil. All survivors were killed at the end of the study. There were fewer tumour-bearing animals among mothers treated with styrene oxide (31%) than among rats given only olive oil (57%); mammary tumours were seen only in the olive-oil-treated animals. The incidences of other tumours were similar. Forestomach tumours were seen only in treated progeny; Carcinomas or early carcinomas of the forestomach occurred in 10/42 males and 16/60 females compared with 1/49 and 1/55 controls respectively. Four tumours of the nervous system occurred in treated groups, whereas only 1 was observed in a male control. Eight lung tumours were seen in treated animals, and 2 in controls; 7 of these tumours (6 malignant + 1 benign) occurred among treated female progeny, giving an incidence of 7/60 (12%) compared with 1/55 (2%) in control females. The incidences of other types of tumour did not show any marked differences between control and treated groups (Ponomarkov et al., 1983; Ponomarkov et al., personal communication).

6.9.3 Summary and conclusions

Of the 6 studies cited, 3 in mice and 3 in rats, only one (O_{20} mice) gave results that indicated a significant increase in lung tumour incidence. The others either did not show any significant increase in tumour incidence or were performed in such a way that conclusions could not be drawn from the results.

The results of studies concerning styrene 7,8-oxide indicated: that there was no development of, or increase in, skin tumours, when the compound was applied to mouse skin; that tumours (benign and malignant) developed in the forestomach, when styrene 7,8-oxide was given by gavage in rats; and that there was an increase in lung tumour incidence in female progeny, when styrene 7,8-oxide was administered to rats pre- and post-natally.

6.10 Genetic effects

Several reviews have been published on the mutagenic and related effects of styrene (Vainio, 1978; IARC, 1979; Norppa, 1981a; Vainio et al., 1982; Zetterberg, 1982; Norppa & Vainio, 1983a).

6.10.1 Chemical reactivity of styrene and styrene oxides

Styrene needs metabolic activation to bind covalently with nucleophilic biological macromolecules. Styrene 7,8-oxide, which has been considered to be a primary mammalian metabolite of styrene (section 5.3), is spontaneously reactive because of the lability of the oxirane ring. Styrene 7,8-oxide can bind covalently with proteins and nucleic acids (Marniemi et al., 1977; van Anda et al., 1979) and is able to alkylate *in vitro* 4-(*p*-nitrobenzyl)-pyridine, a synthetic nucleophile, and deoxyguanosine, a biological nucleophile (Hemminki, 1979; Hemminki & Falck, 1979; Hemminki et al., 1981).

Hemminki (1979) measured the electrophilic reactivity of styrene 7,8-oxide by detecting alkylation of 2-amino-1,7-dihydro-6*H*-purin-6-one (guanine) fluorometrically. The rate of reaction was roughly equal for guanosine, and deoxyguanosine. Styrene 7,8-oxide formed a covalent 7-alkyl derivative with guanine (Hemminki et al., 1980b). With an identical concentration of guanine, RNA and single-stranded DNA were substantially less reactive than guanosine, possibly as a consequence of the tertiary structures of the large relative molecular mass polymers. Double-stranded DNA was even less reactive.

Two highly reactive arene oxide derivatives of styrene, styrene 1,2- and 3,4-oxides were recently synthesized (Hiratsuka & Watabe, 1982; Watabe et al., 1982a,b). These

arene oxides have a very brief half-life and they are very hard to detect, even in an *in vitro* system consisting of styrene, microsomes, and NADPH.

6.10.2 Mutagenic effects of styrene and styrene oxides in bacterial assay systems

Styrene has not been shown to induce reverse mutations in any of the *Salmonella typhimurium* tester strains used in the Ames' test, in the absence of a metabolic activation system (Table 11). However, in the presence of a metabolic activation system, some investigators have found styrene to be mutagenic in the *Salmonella* strains (TA 100, TA 1530, TA 1535) used to detect base-substitution-inducing mutagens; others have obtained negative results (Table 11). No positive results have been reported for styrene in the *Salmonella* strains (TA 98, TA 1537, TA 1538) detecting frame-shift-inducing mutagens. The divergent results on the mutagenicity of styrene in *Salmonella* could be explained, in part, by metabolic differences between the microsomal preparations (S-9 mix, S-10 mix) used. The high volatility, poor solubility, and bacterial toxicity of styrene may also affect the results (de Meester et al., 1981).

Styrene was reported to be positive in the *rec* assay with *Bacillus subtilis* without metabolic activation, in a review by Kawachi et al. (1979), but no specific data were given.

The primary metabolite of styrene, styrene 7,8-oxide, has been shown to be mutagenic in a number of bacterial studies with base-pair strains, without a metabolic activation system (Table 11). Moreover, styrene 7,8-oxide was mutagenic in the fluctuation test with *Escherichia coli* WP2 uvrA (for detecting base-substitution mutagens) and with *Klebsiella pneumoniae* (Hemminki & Falck, 1979; Voogd et al., 1981). The frame-shift mutagen sensitive strains of *Salmonella* gave only negative results with styrene 7,8-oxide, either with or without metabolic activation systems (de Meester et al., 1977; Stolz & Withey, 1977; Drinkwater et al., 1978; Wade et al., 1978; Watabe et al., 1978; Busk, 1979; Hemminki & Falck, 1979; El-Tantawy & Hammock, 1980). Watabe et al. (1981) did not find any appreciable difference in the bacterial (*Salmonella*) mutagenicity of *R*- and *S*-enantiomers of styrene 7,8-oxide and their racemic mixture. However, Pagano et al. (1982) reported that the *R*-enantiomer was more mutagenic to *Salmonella* than the *S*-form, while the racemic styrene 7,8-oxide had an intermediate mutagenic activity.

Table 11. Mutagenicity of styrene and styrene-7,8-oxide in the base-pair substitution strains of Salmonella typhimurium and E. coli [a]

	Results of mutagenicity studies			
	Styrene		styrene 7,8-oxide	
	Metabolic activation system:		Metabolic activation system:	
	None	Present	None	Present
Drinkwater et al. (1978)	..	..	-	-
Glatt et al. (1979)	..	..	+	..
Hemminki & Falck (1979)	..	..	+	..
Pagano et al. (1982)	..	..	+	..
Planche et al. (1979)	..	..	+	..
Sugiura & Goto (1981)	..	..	+	..
Sugiura et al. (1978a, b)	..	..	+	..
Turchi et al. (1981)	..	..	+	..
Voogd et al. (1981)	..	..	+	..
Wade et al. (1978)	..	..	+	..
Watabe et al. (1981a)	..	..	+	..
El-Tantawy & Hammock (1980)	..	..	+	+[b]
Yoshikawa et al. (1980)	..	..	+	+[b]
Kawachi et al. (1979)	-	-	..	..
Milvy & Garro (1976)	-	..	+	..
Greim et al. (1977)	-	-	+	..
Busk (1979)	-	-	+	+[b]
Loprieno et al. (1978) Stolz & Withey (1977)	-	-	+	+
Watabe et al. (1981, 1982b)	-	+[c]	+	-
de Meester et al. (1977, 1981)	-	+	+	+[b]
Vainio et al. (1976)	-[d]	+	+	+[b]
Poncelet et al. (1980)	-	+	..	..
Cerna & Kypenova (1977)	..	+[e]	..	..

.. No data.

[a] Modified from: Norppa (1981a).

[b] A decrease reported in mutagenic activity, as compared to treatment without metabolic activation.

[c] Only with trichloropropane oxide present.

[d] A weak activity reported also without S-9 mix in TA 1535.

[e] Host mediated assay, Salmonella strain TA 1950, mice as hosts.

Two arene oxide derivatives of styrene, styrene 1,2- and 3,4-oxide, both of which have been considered to be the precursors of isolated urinary metabolites of styrene in the rat, have been demonstrated to be mutagenic towards a base-pair strain (TA 100) of Salmonella, but not towards a frame-shift strain (TA 98) (Watabe et al., 1982b). The 1,2- and 3,4-oxides were more mutagenic than the 7,8-oxide, but only after sequential addition to the medium during pre-incubation.

Thus, the results obtained with various bacterial assay systems show that styrene 7,8-oxide is a direct base-pair substitution type mutagen. The extremely labile minor metabolites of styrene, 1,2- and 3,4-oxide, seem to be highly reactive and are mutagenic for Salmonella. However, with styrene, even in the presence of a metabolic activation system, contradictory results have been reported.

6.10.3 Genetic effects of styrene and styrene 7,8-oxide in eukaryotic non-mammalian systems

Various non-mammalian assay systems, including point mutations and gene conversion in yeast, point mutations in silk worm, recessive lethal mutations, sex chromosome loss and non-disjunction in Drosophila melanogaster, and chromosome damage in root tip cells of Allium cepa, have been applied to study the mutagenic and genetic effects of styrene and styrene 7,8-oxide.

In the yeast assays, styrene did not induce mitotic gene conversions at the adenine and tryptophane loci of Saccharomyces cerevisiae or forward mutations at the adenine loci of Schizosaccharomyces pombe, even in the presence of a mouse liver microsome mix (Loprieno et al., 1976; Loprieno, 1981; Loprieno & Abbondandolo, 1980). According to Bauer et al. (1980, 1981), this negative result could be because the epoxide hydrotase (EC 4.2.1.63) (inactivation enzyme) is more stable than the monooxygenase, because the concentration of styrene 7,8-oxide in the incubation mixture could not reach a mutagenic level. When a yeast strain (Saccharomyces cerevisiae D 7) capable of metabolic activation was used to test the induction of gene conversions by styrene, a positive result was reported (Del Carratore et al., 1982). Styrene 7,8-oxide has been found positive in all the yeast systems (Loprieno et al., 1978; Loprieno, 1981).

When tested in a host-mediated assay, with mice as the host animal and yeast as the target cells, styrene and styrene 7,8-oxide were weakly mutagenic for some genetic end points (Loprieno et al., 1978), but these results were later considered negative for styrene (Loprieno & Abbondandolo, 1980) when a higher number of historical control values became available.

Table 12. Point mutations, chromosome aberrations and genetic damage induced in cultured mammalian cells by styrene and styrene 7,8-oxide

Test-system	Type of damage assayed	Styrene		Styrene 7,8-oxide		References
		Metabolic activation system: None	Present	Metabolic activation system: None	Present	
Rodent cell lines						
V79	HGPRT mutations	..	..	+	..	Bonatti et al. (1978); Sugiura et al. (1979)
		-	-	+	..	Loprieno et al. (1976, 1978)
		-	+	+	-[c]	Beije & Jenssen (1982)
	aberrations	..	..	+	..	Turchi et al. (1981)
	micronuclei	..	..	+	..	
	anaphase bridges	..	..	+	..	
CHL	aberrations	-	+[a]	+	..	Ishidate & Yoshikawa (1980); Ishidate et al. (1981); Kawachi et al. (1979); Matsuoka et al. (1979)
CHO	SCEs	-	+[b]	+	+[d]	de Raat (1978)
		..	..	+	..	Kubiak et al. (1981)
L5178Y	TK mutations	..	..	+	-	Amacher & Turner (1982)
Human cells						
Lymphocytes	aberrations	+[e]	..	+	..	Linnainmaa et al. (1978a, b);
	micronuclei	+[e]	..	+	..	Meretoja & Vainio (1979)
	aberrations	..	..	+	..	Fabry et al. (1978)

Table 12 (contd)

Test-system	Type of damage assayed	Styrene		Styrene 7,8-oxide		References
		Metabolic activation system: None	Present	Metabolic activation system: None	Present	
	aberrations	+[e]	..	+	..	Norppa et al.(1980a, 1981, 1982);
	SCEs	+[e]	..	+	..	Norppa (1981a); Norppa & Vainio (1983b)
	unscheduled DNA synthesis	-[f]	..	..	..	Pero et al. (1982)
E UE	unscheduled DNA synthesis	..	-	+	..	Loprieno et al. (1978)
Wistar rat hepatocytes						
	unscheduled DNA synthesis	..	..	..	-	Brouns et al. (1979)

.. No data.
[a] With methylcholantrene preinduced rat liver S-9.
[b] Only with cyclohexene oxide present.
[c] In the liver perfusion system.
[d] A decrease in activity.
[e] In vitro activation by erythrocytes suggested.
[f] Purified lymphocytes.

In Drosophila, a significant increase in the frequency of X-linked recessive lethals was observed in the offspring of males fed styrene or styrene 7,8-oxide (Donner et al., 1979). The test for sex chromosome loss and non-disjunction in Drosophila gave negative results for styrene (Penttilä et al., 1980). In a review (Kawachi et al., 1979), styrene was reported not to induce point mutations in silk worm.

Results of cytogenetic studies in meristematic root cells of Allium cepa demonstrated that styrene induces metaphase chromosome breaks and micronuclei (Linnainmaa et al., 1978a, b). Styrene 7,8-oxide induced micronuclei and anaphase fragments and bridges in these growing root-tip cells (Linnainmaa et al., 1978a, b).

6.10.4 Genetic effects of styrene and styrene 7,8-oxide in mammalian cells in vitro

Point mutations, chromosome damage, and unscheduled DNA synthesis (UDS) have been studied after treatment of mammalian cell cultures with styrene or styrene 7,8-oxide (Table 12).

In the Chinese hamster V79 cell line, styrene did not induce point mutations at the HPRT locus, with or without a metabolic activation system (S-10 mix) from mouse liver (Loprieno et al., 1976, 1978; Loprieno, 1981). On the other hand, Beije & Jenssen (1982) found styrene weakly mutagenic in the same system after metabolic activation by rat liver S-9 mix. When a liver perfusion system was used for the metabolic activation of styrene, there was a clear increase in point mutations at the HRPT locus of V79 cells (Beije & Jenssen, 1982).

Loprieno et al. (1978) examined the effects of styrene (with mouse liver S-10 mix) and styrene 7,8-oxide on UDS in human heteroploid EUE cell line. There was an increase in UDS with styrene 7,8-oxide but not with styrene. Brouns et al. (1979) used freshly isolated hepatocytes of Wistar rats for UDS detection after styrene 7,8-oxide treatment. The results were negative, which according to the authors was due to the rapid inactivation of styrene 7,8-oxide in the hepatocytes. Pero et al. (1982) did not find any effects of styrene (without metabolic activation) on UDS in purified resting human lymphocytes.

Styrene induced chromosome aberrations, micronuclei, and SCEs in human whole blood lymphocyte cultures, and chromosome aberrations in Chinese hamster CHL cells in the presence of metabolic activation (Table 12). Styrene 7,8-oxide induced chromosome aberrations, micronuclei, and SCEs, in rodent cell lines and in human lymphocyte cultures without metabolizing systems (Table 12). The positive effects of styrene in human whole blood lymphocyte cultures are explained by the ability

of erythrocytes to convert this compound into styrene 7,8-oxide (Norppa et al., 1980a; Belvedere & Tursi, 1981; Norppa et al., 1982; Vainio et al., 1982).

Without metabolizing systems, styrene 7,8-oxide has been clearly shown to induce HGPRT mutations in V79 cells (Loprieno et al., 1976; 1978; Bonatti et al., 1978; Sugiura et al., 1979; Loprieno & Abbondandolo, 1980; Loprieno, 1981; Beije & Jenssen, 1982) and point mutations in the TK locus of the mouse lymphoma cell line L5178 Y (Amacher & Turner, 1982). With the liver perfusion technique, styrene 7,8-oxide did not induce point mutations at the HPRT locus of V-79 cells (Beije & Jenssen, 1982).

6.10.5 Genetic effects of styrene and styrene 7,8-oxide in mammalian systems in vivo

Styrene and styrene 7,8-oxide have been tested in vivo for their ability to induce genetic damage in somatic and germ cells in different species (mouse, rat, Chinese hamster) using different genetic endpoints (chromosome aberrations, micronuclei, dominant lethals, SCEs and translocations in spermatocytes; Table 13). Most of the studies concerning chromosome aberrations in the bone marrow cells of animals were negative for styrene. No increases in chromosome aberrations in bone marrow were observed in mice after high single or repeated doses of styrene administered by gavage or intraperitoneally (i.p.), or in rats after an unspecified dose. However, a positive effect for chromosome aberrations in rat bone marrow was found after inhalation exposure, and for SCEs in mouse bone marrow, alveolar macrophages, and regenerating liver cells. In the micronucleus test, styrene gave negative results in Chinese hamsters but positive results in mice (Penttilä et al., 1980; Norppa, 1981b).

Studies on the cytogenetic effects of styrene 7,8-oxide in whole mammals also gave contradictory results (Table 13). Loprieno et al. (1978) observed a dose-dependent clastogenic effect in mouse bone marrow cells after oral administration of styrene 7,8-oxide. However, Fabry et al. (1978) did not find any chromosome aberrations or micronuclei in the bone marrow of mice after an i.p. administration. Penttilä et al. (1980) also failed to detect any effects on micronuclei in Chinese hamster bone marrow erythrocytes after an i.p. administration. Negative findings were reported by Norppa et al. (1979) for chromosome aberrations and SCEs in the bone marrow cells of Chinese hamsters after inhalation exposure. A slight increase in SCEs was observed after i.p. injection of a lethal dose; chromosomal aberrations were also elevated. Conner et al. (1982) noticed a slight increase in SCEs in regenerating liver

Table 13. Cytogenetic damage induced by styrene and styrene 7,8-oxide in various rodent studies in vivo

Species (strain) Cell type	Exposure: Route[a]	Exposure: Dose	Exposure: Duration of time after treatment	Type of damage studied	Result	Reference
STYRENE						
Rat (Wistar)						
bone marrow	inhalation	1260 mg/m³ (300 ppm)	9-11 weeks	Chromosomal aberrations	+	Meretoja et al. (1978b)
(Wistar)						
bone marrow	inhalation	..	..	Chromosomal aberrations	-	Kawachi et al. (1979)
Mouse (BDF_1)						
bone marrow alveolar macrophages, regenerating	inhalation	1625-3872 mg/m³ (387-922 ppm)	4 days	SCEs	+	Conner et al. (1979; 1980; 1982)
liver cells		3872 mg/m³ (922 ppm)	2 days	SCEs	+	
(C57BL/6)						
polychromatic erythrocytes	i.p.	250 or 1000 mg/kg	30 h	Micronuclei	+	Norppa (1981b)
		500 or 1500 mg/kg	30 h	Micronuclei	-	
(CD-1)						
bone marrow	p.o.	500 or 1000 mg/kg	24 h	Chromosomal aberrations	-	Loprieno et al. (1978)
	p.o.	4 x 500 mg/kg	4 days	Chromosomal aberrations	-	Sbrana et al. (1982)
		70 x 200 mg/kg	70 days	Chromosomal aberrations	-	
(ICR)						
bone Marrow	i.p.	$\frac{LD^{50}}{2}$ or $\frac{5xLD^{50}}{6}$	6, 24, or 48 h	Chromosomal aberrations	-	Cerna & Kypenova (1977)
Chinese hamster						
bone marrow	inhalation	1260 mg/m³ (300 ppm)	4 or 21 days	Chromosomal aberrations	-	Norppa et al. (1980b)
polychromatic erythrocytes	i.p.	1000 mg/kg	30 h	Micronuclei	-	Penttilä et al. (1980)

Table 13 (contd).

Species (strain) Cell type	Exposure: Route[a]	Exposure: Dose	Duration of time after treatment	Type of damage studied	Result	Reference
STYRENE 7,8-OXIDE						
Mouse						
(BDF_1)						
bone marrow, alveolar macrophages, regenerating liver cells	inhalation	210 mg/m³ (50 ppm)	5 h	SCEs	+[b]	Conner et al. (1982)
(CD-1)						
bone marrow	p.o.	500-1000 mg/kg	24 h	Chromosomal aberrations	+	Loprieno et al. (1978)
(BALB/c)						
bone marrow	i.p.	250 mg/kg	1-13 days	Chromosomal aberrations	-	Fabry et al. (1978)
polychromatid erythrocytes			30 h	Micronuclei	-	
primary spermatocytes			2.5-3 months[c]	Trans-locations	-	
post-meiotic sperm cells			1-3 weeks	Dominant lethals	-	
Chinese hamster						
bone marrow	inhalation	105-420 mg/m³ (25-100 ppm)	2,4, or 20 days	Chromosomal aberrations	-	Norppa et al. (1979)
				SCEs	-	
	i.p.	500 mg/kg	24 h	Chromosomal aberrations	-[d]	
			7 h	SCEs	+[e]	

.. No data.
a i.p. = intraperitoneal, p.o. = peroral.
b Negative in bone marrow.
c Considered to be too long time for a positive result.
d Positive in animals who died of the treatment.
e A slight effect.

cells and alveolar macrophages (but not in bone marrow) in hepatectomized mice exposed through inhalation to styrene 7,8-oxide.

Only one study has appeared concerning the possible effects of styrene 7,8-oxide on germinal cells. Fabry et al. (1978) reported negative results for translocations in primary spermatocytes after a single i.p. injection and for dominant lethals in male postmeiotic germ cells of mice.

In summary, the results concerning the genetic effects of styrene and styrene 7,8-oxide in vivo are conflicting. Positive results for both compounds have been obtained mainly at toxic or nearly toxic doses. The studies have dealt with different indicators of chromosome damage (aberrations, micronuclei, SCEs) in actively dividing somatic cells. The effects of styrene 7,8-oxide on germ cells have been examined only in one study, with negative results. On the basis of the activity of monooxygenase and epoxide hydrolase in different species, mice would be expected to be more sensitive to styrene than rats or Chinese hamsters (Cantoni et al., 1978; Norppa et al., 1979; Norppa, 1981a). Even in mice, styrene and styrene 7,8-oxide disappeared rather rapidly after intraperitoneal or oral administration (Bidoli et al., 1980; Pantorotto et al., 1980; Sbrana et al., 1982). However, the results of the mutagenicity studies available cannot at present be interpreted solely according to such differences in metabolism.

6.10.6 Conclusions on the genetic effects of styrene

Styrene is a potential mutagen only after metabolic activation. Its most important active metabolite seems to be styrene 7,8-oxide. Arene oxides (styrene 1,2-oxide and styrene 3,4-oxide) may also be of importance, but at present their role cannot be evaluated.

Genetic effect tests in bacteria, yeasts, and mammalian cells in vitro have yielded contradictory results for styrene, in the presence of microsomal fraction of rodent liver (usually S-9 mix). This appears to be mostly due to differences in the efficiency of activation of styrene in different studies.

Results of in vivo studies on the genetic effects of styrene in mammals are also contradictory. Some of the discrepancies can be explained by variations in metabolic capacity among the rodent species used, divergence in dose levels, and by the different routes of administration.

Styrene 7,8-oxide is a direct mutagen that is clearly positive in vitro without metabolic activation, but gives contradictory results in mammals in vivo.

6.11 Effects on Reproductive Function and Teratogenic Effects

(a) Non-mammalian systems

Styrene and styrene 7,8-oxide have been reported to be embryotoxic and teratogenic in chick embryos (Vainio et al., 1977). Kankaanpää et al. (1979) found that trichloropropylene oxide increased the embryo toxicity and teratogenicity of styrene and styrene oxide. The LD_{50}s were 40 µmol/egg for styrene and 1.5 µmol/egg for styrene oxide. Depending on the dose, malformations were found in up to 20% of the test embryos but not in the controls. The types of malformation included: absence of one or both eyes, eyelid deformation, deformation of the skull, exencephaly, exteriorization of viscera, etc. Styrene and styrene oxide have also been found to interfere with the development of sea urchin embryos (Pagano et al., 1978).

(b) Mammalian systems

When male rats were exposed to a styrene concentration of 200 mg/m^3 (48 ppm) for 5 h/day, 5 days per week for 4 months, Ivanova-Tchemichanska et al. (1982) found decreased osmotic resistance and mobility of spermatozoa, decreased sulfhydryl content, and desquamated epithelium in the seminiferous tubules.

Ragyl'ye (1974) studied the effects of styrene on pregnant rats exposed to 1.47, 5, and 50 mg/m^3 (0.35, 1.2, and 12 ppm) for 4 h/day throughout gestation. Resorptions were noted at all exposure levels but the number of malformations was not significantly increased. Murray et al. (1978) exposed rats and rabbits to styrene through inhalation (1260 and 2520 mg/m^3; 300 and 600 ppm) for 7 h/day from day 6 to 15 (rats) or from day 6 to 18 (rabbits), and by gavage (in peanut oil, 180 and 300 mg/kg body weight/day). No increase in the incidence of resorptions or major malformations, compared with controls, was reported. Delayed ossification was noted in rabbits exposed to 2520 mg/m^3 (600 ppm). In an inhalation study, Vergieva et al. (1979) exposed rats to styrene at 200 and 400 mg/m^3 (48 and 96 ppm) for 4 h daily, 5 days a week, throughout pregnancy. There was not any evidence of embryotoxicity or teratogenicity. Mice were exposed to a styrene level of 1050 mg/m^3 (250 ppm) and Chinese hamsters to 1260, 2100, 3150, and 4200 mg/m^3 (300, 500, 750, and 1000 ppm) for 6 h daily on days 6-16 (18 for hamsters) of gestation. An increased number of resorptions was noted in both species at the highest concentration tested. A slight increase (not statistically significant) was observed in the

number of minor skeletal malformations (rib fusion, extra ribs) in mice but not in hamsters (Kankaanpää et al., 1980).

Quast et al. (1978) exposed pregnant Sprague-Dawley rats and New Zealand white rabbits to styrene concentrations of 1260 or 2520 mg/m^3 (300 or 600 ppm) for 7 h/day on days 6-15 (rats) and 6-10 (rabbits) of gestation. Additional groups of rats were given styrene (90 or 150 mg/kg body weight daily) on days 6-15 of gestation. No teratogenic effects were detected.

In a 3-generation reproduction study in rats, styrene was administered in the drinking-water at 125 and 250 mg/litre (estimated daily intake 14 and 21 mg/kg body weight per day for males and females, respectively, at the high dose). No adverse effects on reproductive capacity were observed. Some significant effects on early survival in the second generation offspring at the high dose were found in 2 specific litters. No further evaluation could be made, since data for individuals were not available (Litton Bionetics, 1980).

7. EFFECTS OF STYRENE IN MAN

7.1 Controlled Human Studies

Few controlled exposure studies have been reported in which the effects of styrene on physiological functions in man have been investigated. Attention has been focused on the irritant effects of styrene vapour on the mucous membranes and on the central nervous system effects caused by styrene inhalation.

The odour perception threshold for styrene in air was determined by Smith & Hochstettler (1969) to be as low as 0.2-0.34 mg/m^3 (0.05-0.08 ppm). At higher concentrations, the odour of styrene was clearly perceptible and it was reported as "strong but not objectionable" at about 420 mg/m^3 (100 ppm) (Stewart et al., 1968). The ability to detect the odour typically fades as the exposed individuals become adapted (Stewart et al., 1968). Styrene vapour was irritating to the eye and the nose at concentrations exceeding 840 mg/m^3 (200 ppm) and, when the styrene concentration approached 1596 mg/m^3 (380 ppm), it was poorly tolerated (Stewart et al., 1968). Strong immediate irritation of the eyes and the respiratory tract has been reported at styrene concentrations in air of 2520-3360 mg/m^3 (600-800 ppm) (Carpenter et al., 1944; Wolf et al., 1956).

Oltramare et al. (1974) exposed 6 volunteers to styrene at 210, 420, and 840 mg/m^3 (50, 100, and 200 ppm) over 1-3 h and noted that for a combination of symptoms of the mucous membrane (irritation of eyes, nose, and lips) and central nervous system (dizziness, headache, drowsiness, difficulty in concentrating, lightheadedness, fatigue), the number of symptoms increased with dose. This increase was still evident at the 210 mg/m^3 (50 ppm level). At the 2 higher levels, digestive disturbances also occurred. In studies by Gamberale & Hultengren (1974), 12 volunteer subjects were exposed to styrene vapour through a mouth tube at concentrations increasing stepwise 210, 630, 1050, and 1470 mg/m^3 (50, 150, 250, and 350 ppm), each successive step lasting about 30 min. When the volunteers were asked afterwards to rate their subjective sensations (during the exposure) it appeared that exposure to styrene had made them feel more tense and more "affected" compared with control conditions. The authors also studied the psycho- physiological performance of the subjects. Simple reaction times tended to be prolonged with increasing exposure. The changes became statistically significant during the final 30 min, when the environmental concentration reached 1470 mg/m^3 (350 ppm). Tests on perceptual speed and manual dexterity did not show any impairment in relation to styrene exposure.

Stewart et al. (1968), however, noted an impairment in performance of tasks involving manual dexterity and eye-hand coordination in some subjects exposed to a styrene concentration of about 1596 mg/m³ (380 ppm) over a 1-h period. On the basis of a study on 3 volunteers, Oltramare et al. (1974) reported impaired reaction time to visual stimuli, to combined visual-acoustic stimuli, and in a test of diffuse attention during and immediately after exposure to styrene at 210, 420, and 840 mg/m³ (50, 100, and 200 ppm) for 1.5 h. There was no clear dose dependance. Inhalation exposure to high concentrations of styrene (3360 mg/m³; 800 ppm) caused symptoms of impairment of balance in 2 subjects and a marked unsteadiness in posture was observed (Carpenter et al., 1944). At a styrene level of about 1596 mg/m³ (380 ppm) for 1 h, 2 out of 5 subjects showed abnormal results in the modified Romberg test indicating difficulty in maintaining balance. Furthermore, after a 7-h exposure to about 420 mg/m³ (100 ppm), 3 out of six subjects reported that they had transitory difficulty in performing the modified Romberg test, though no objective signs of impairment of balance were found (Stewart et al., 1968). Oltramare et al. (1974), in a study on 3 subjects, showed impairment of balance on a body sway platform at a styrene concentration of 840 mg/m³ (200 ppm) but not at 420 mg/m³ (100 ppm).

Five volunteers were exposed by Ödkvist et al. (1980) to styrene at about 1260 mg/m³ (300 ppm) for 1 h, using a mouth tube. Treatment was combined with exercise on a bicycle ergometer with a 50 W workload, and observations were made on both equilibrium ability and functioning of the vestibular system immediately after exposure. None of the subjects exhibited spontaneous, fixation or positional nystagmus, whereas an impairment of eye-tracking ability was found in all individuals in an optokinetic test. The difference in eye-movement changes between styrene-exposed and control groups was not statistically significant. No styrene-induced changes were found in conventional clinical tests of balance (standing on one leg with eyes closed, walking a line with eyes closed). The results were thought to suggest that exposure to styrene (inducing blood levels of approximately 80 µmol/litre) decreased the inhibitory effect of the cerebellum on the motor function of the eyes.

In summary, the odour threshold for styrene was found to be 0.2-0.34 mg/m³ (0.05-0.08 ppm) and the odour was uncomfortable at elevated concentrations. Styrene induced subjective symptoms of irritation of the mucous membranes at concentrations exceeding 420-840 mg/m³ (100-200 ppm). In the same concentration range, subjective symptoms of the central nervous system, such as dizziness, lightheadedness, headache,

and drowsiness may occur. Reaction time, performance, and body balance tend to be impaired by short-term inhalation exposure to styrene at concentrations of 630-840 mg/m³ (150-200 ppm) and definite impairment occurs at concentrations exceeding 1470 mg/m³ (350 ppm).

7.2 Epidemiological Studies

7.2.1 Haematopoietic and immune system

Chmielewski & Renke (1975), who studied a group of 101 workers exposed for at least one year to a styrene concentration of 100-300 mg/m³ (24-72 ppm), did not demonstrate any appreciable effects on the haemoglobin concentration, erythrocyte count, leukocyte count, or differential count. Workers exposed for more than 10 years, however, had a slightly decreased thrombocyte count compared with workers exposed for shorter periods.

In a study on 494 workers at different levels and durations of exposure, Lorimer et al. (1976) observed a random distribution of abnormal haemoglobin concentrations, and leukocyte or platelet counts between the groups. Thiess & Friedheim (1978), who investigated 84 workers exposed to 210-2100 mg/m³ (50-500 ppm) for 1-36 years, did not notice any appreciable differences in haemoglobin concentrations, or leukocyte, erythrocyte, or platelet counts compared with a reference group.

In the studies of Chmielewski & Renke (1975), and Renke & Chmielewski (1976), 20 styrene workers did not show any differences in bleeding time or fibrinogen level compared with a reference group. However, the coagulation time and the adhesivity of the platelets were somewhat increased in the styrene-exposed group and the prothrombin index was slightly reduced.

There is very little information on the immunological effects of styrene. In the immunophoresis study of Chmielewski et al. (1973), no dose-related differences were observed in concentrations of serum gamma globulin among workers exposed to different concentrations of styrene.

7.2.2 Nervous system

Four studies have dealt with reaction times among workers occupationally exposed to styrene. One study (Götell et al., 1972) in which 17 men were exposed to a styrene concentration of 630 mg/m³ (150 ppm) showed prolonged simple reaction times in the styrene-exposed workers, both in the morning and in the afternoon, compared with an age-matched control group. A second study of 106 workers in 4 work places indicated

longer and more irregular reaction times in workers exposed to styrene than in controls (Gamberale et al., 1975). The differences were still present after a night's rest. The mean styrene concentration determined by continuous measurement in the workers' breathing zone was 57-426 mg/m³ (13.6-101.4 ppm). The mean duration of exposure was 2.7 years (range 0.1-11.0 years).

Another study with a similar study design, also revealed prolonged reaction times during the working day among styrene- and acetone-exposed boat manufacturers (Kjellberg et al., 1979). The exposed group (7 workers, average styrene concentration 37 mg/m³ (9 ppm), acetone concentration 82 mg/m³, mean employment time 10.5 years) did not show any deterioration in sensory motor functions in relation to styrene exposure and the addition task. There was a correlation between the reaction-time impairment and the total uptake of styrene divided by the estimated amount of adipose tissue.

In the study of Kjellberg et al. (1979), the reaction times of 3 subjects were followed after removal from exposure. Reaction times improved after 4 days and there was a further improvement after 35 days.

The study by Cherry et al. (1980) dealt with 27 workers (mean age, 23 years), who were exposed to a time-weighted average level of styrene of 386 mg/m³ (92 ppm). Among the psychological and behavioural tests applied, differences between the exposed and unexposed groups were seen only in reaction times.

Several case reports have described neurasthenic symptoms among patients who had been occupationally exposed to styrene (Klimkova-Deutschova, 1962; Axelson et al., 1974; Spasovski et al., 1976).

A considerable percentage of 101 workers employed in the polyester laminate industry had disturbances of the nervous system, particularly of the vegetative nervous system. The exposure level of the workers ranged from approximately 100 to 300 mg/m³ (24 to 72 ppm). The urinary mandelic acid excretion was more than 400 mg/litre in 36 of the workers (Chmielewski et al., 1973).

Schneider & Seeber (1979) used psychological tests on 2 groups occupationally exposed to styrene, one group (35 persons) having a mean exposure of 700 mg/m³ (168 ppm), and the other (46 persons), a mean exposure of 300 mg/m³ (72 ppm). Exposure was associated with poor visual attention, interferences with attention span, perceptual inaccuracy, and decreased manual dexterity.

In studies concerning 98 male laminating workers having a mean urinary mandelic acid concentration that varied from 7 to 4715 mg/litre (median concentration 808 mg/litre), symptoms of

tiredness, and difficulty in concentrating were mentioned more often than among a reference population (Härkönen, 1977). The duration of styrene exposure varied from 0.5 to 14 years (median 5.1 years). In psychological tests, the same styrene-exposed workers showed visuomotor inaccuracy and poor psychomotor performance (Lindström et al., 1976). Exposure-effect relationships were drawn between the urinary mandelic acid concentration and a combined score for 3 tests (symmetry drawing, the Bourdon-Wiersma test, and the Mira test). Analysis of the exposure-response relationship for these tests showed a significant increase in response for workers with levels of urinary mandelic acid exceeding 1600 mg/litre (75% having mandelic acid levels of more than 2000 mg/litre) (Härkönen et al., 1978). The proportion of abnormal EEGs also increased with increasing exposure levels (Seppäläinen & Härkönen, 1976). Workers with urinary mandelic acid concentrations above 700 mg/litre had significantly ($P < 0.05$) more frequent abnormal EEGs than those with lower levels (Seppäläinen & Härkönen, 1976; Härkönen et al., 1978).

EEG abnormalities have been reported to be more prevalent among young styrene workers, especially during the first years of exposure (Dolmierski et al., 1974; Chmielewski et al., 1977). EEG abnormalities, indicated mainly by increased slow activity in the theta range, did not increase with longer exposure (Roth & Klimkova-Deutschova, 1963; Seppäläinen & Härkönen, 1976), though paroxysmal episodes have also been evident (Dolmierski et al., 1974; Seppäläinen & Härkönen, 1976). Results of a study by Rosén et al. (1978) in which 33 men were exposed to styrene concentrations ranging from less than 21 mg/m^3 (5 ppm) to 735 mg/m^3 (175 ppm), showed an increased beta activity in 9 subjects and slow activity (theta waves) in 6. The EEG abnormalities were non-specific and resembled those caused by various other solvents (Rosén et al., 1978; Seppäläinen et al., 1980).

Styrene exposure may also affect peripheral nerves (Matsushita et al., 1968, Lilis et al., 1978; Rosén et al., 1978). Lilis et al. (1978) found distal hypoaesthesia in the lower extremities of 412 styrene-exposed workers (persons with diabetes mellitus, a history of back injury, and/or excess alcohol intake were excluded). The frequency of this symptom varied from 4.1% among those exposed for less than 7 years to 8.5% among those exposed for more than 20 years. Abnormally low motor conduction velocity (MCV) of the radial nerve was found in 15 out of 80 persons tested and a slowed MCV of the peroneal nerve in 12 out of 73 tested. The MCV of the peroneal nerve decreased with increasing length of exposure, but the effect of age was not considered. However, the level of exposure did not have any effect on the peroneal nerve conduction. The MCV of the radial nerve was not correlated

with the length or level of exposure. Rosén et al. (1978) did not find differences in the MCVs of the median, ulnar, peroneal, or posterior tibial nerves of 33 workers exposed to styrene, compared with normal controls. However, the amplitude of the sensory action potential of the median nerve was reduced and the duration of this potential increased among styrene-exposed workers, even in 10 individuals who had been exposed to styrene levels of less than 21 mg/m³ (5 ppm). The dose-effect relationships were conflicting. Disorders in attention span and movement coordination in psychological tests were reported in 12 out of 102 workers, occupationally exposed to styrene concentrations of 10-100 mg/m³, who had urinary mandelic acid concentrations of 30.9-268 g/kg creatinine (Spasovski et al., 1980). The same authors also reported diminished alcohol tolerance among styrene workers.

In summary, the results of 3 studies showed that simple reaction times were prolonged in workers occupationally exposed to styrene at levels below 630 mg/m³(150 ppm), while one study gave equivocal results at a time-weighted average level of styrene in air of 386 mg/m³ (92 ppm). Another study suggested that the effect on reaction time was reversible.

Surveys of styrene-exposed workers have shown that an increased incidence of abnormalities in electroencephalographic recordings at mean styrene levels of less than 420 mg/m³ (100 ppm) was related to styrene exposure levels. Some slight disturbances in visuomotor accuracy and psychomotor performance were noted in workers exposed to levels of the order of 210 mg/m³ (50 ppm) or more. Various neurasthenic and autonomic symptoms were also reported among these workers.

7.2.3 Kidneys and the urinary tract

There are only a few studies of the effects of styrene exposure on the human kidney. Härkönen (1977), who studied laminating workers exposed to styrene for periods ranging from 0.5 to 14 years, could not find any cytological changes differing from Papanicolau I in 35 urine specimens. Results of studies concerning mortality and morbidity rates due to kidney and urinary tract disorders have not revealed any differences between subjects exposed to styrene and unexposed subjects (Lorimer et al., 1978; Thiess & Friedheim, 1978).

In studies on organic solvent exposure in relation to kidney function, Askergren et al. (1981) reported that workers exposed to organic solvents, especially to styrene, excreted significantly larger quantities of albumin in the urine than unexposed workers. No differences were observed in beta-2-microglobulin excretion. The same author compared the

excretion of erythrocytes and leukocytes in the urine of 101 men, occupationally exposed to styrene or toluene or to a combination of xylene and toluene, with the cell excretion in the urine from 39 unexposed controls. While no changes in glomerular filtration rates were observed, the urine of exposed men contained significantly more cells than that of the controls.

7.2.4 Gastrointestinal tract

Baširov (1975) reported studies on the digestive system of 130 workers (89 men, 41 women) engaged in styrene- butadiene synthetic rubber manufacture. The workers were mostly 20-40 years old and the length of exposure varied from less than 5 years (16 workers) to more than 10 years (28 workers). Average styrene concentrations were 60-130 mg/m^3 (14-31 ppm). Tests of secretory, excretory, motor, and pepsinogen-generating functions of the stomach were conducted on 20 unexposed people and on 80 workers who first developed symptoms related to the digestive system after working in the plant. Thirty-six had decreased digestive function, 25 had decreased peristalsis, and 51 had decreased acidity. In further studies, chronic gastritis was diagnosed in 35 of these workers.

7.2.5 Liver

Lorimer et al. (1976) assessed the liver function of 493 styrene-exposed workers. Exposure concentrations were not measured. The activities of alkaline phosphatase (AP) (EC 3.1.3.1), aspartate aminotransferase (ASAT or GOT) (EC 2.6.1.1), alanine aminotransferase (ALAT or GPT) (EC 2.6.1.2), and gamma glutamyltranspeptidase (γ-GTP) (EC 2.3.2.1) and the amount of serum bilirubin were determined. Only the γ-GTP activity showed a significant elevation in the high-exposure group compared with the low-exposure category, even when alcohol intake was taken into account (1.7% versus 7.2%; $0.01 < P < 0.02$).

Axelson & Gustavson (1978) gathered data on ASAT, ALAT in serum from 35 styrene-exposed male workers and 12 unexposed controls. The time-weighted average exposure levels were less than or about 420 mg/m^3 (100 ppm). The average transferase levels were higher among men who had been exposed, but only ASAT was significantly elevated ($P < 0.001$). No differences were found in the average levels of AP.

The serum levels of ALATP, ASAT, γ-GTP, LDH, and SDH (sorbitol dehydrogenase (EC 1.1.1.14)) enzymes were studied in 72 workers exposed to styrene in the manufacture of plastic boats. The exposure levels varied between very low

concentrations < 21 mg/m³ (< 5 ppm) and about 170 mg/m³ (40.8 ppm). The styrene-exposed groups had a higher mean γ-GTP value than the control group ($P < 0.05$). However, there was no dose dependence (Lundberg, 1981).

Vihko et al. (1983) studied 25 persons exposed to styrene at about 126-168 mg/m³ (30-40 ppm) (mean exposure time 3 years, all exposures exceeding 1 year). The serum activities of ALAT, ASAT, γ-GTP, and LD and the concentrations of serum total bilirubin and conjugated bilirubin were determined, as well as serum bile acids, cholic acid, and chenodeoxycholic acid. An elevated concentration of chenodeoxycholic acid in serum was the most frequently found parameter among styrene-exposed workers. Spasovski (1976) showed that the serum protein profile changed and that serum transaminase activity was elevated at a styrene concentration of 2000 mg/m³ (500 ppm).

In conclusion, a clear-cut trend towards altered liver function was not demonstrated. At low exposure levels, the commonly used parameters such as serum activity of enzymes of hepatic origin have given equivocal results.

7.2.6 Cardiovascular system

The thorax radiographs of 84 workers were evaluated before the beginning of employment and after exposure to styrene at 210-1260 mg/m³ (50-300 ppm) for 1-36 years (Thiess & Friedheim, 1978). The authors could not attribute any grossly observable changes to styrene exposure, nor did they observe any "gross pathological indications in the electrocardiograms" compared with those of a reference group of 62 subjects.

7.2.7 Respiratory system

In clinical studies, Wilson (1944) found that styrene-exposed workers reported irritation of the eyes, throat, and respiratory tract. It has frequently been reported that people in areas of high styrene exposure complain of irritation of the eyes and nasopharynx. Götell et al. (1972) examined 15 workers occupationally exposed to a time-weighted average styrene concentration of 71-1218 mg/m³ (17-290 ppm) and found that lung function tests (forced vital capacity and forced expiratory volume in 1 s) were normal and did not change during the working day. However, the concentrations of styrene that gave rise to complaints varied from person to person, apparently depending on individual tolerance. In a study on styrene-exposed workers who had respiratory tract symptoms, it was suggested that long-term exposure to styrene could cause obstructive pulmonary changes (Chmielewski & Renke, 1975).

In a cross-sectional study of clinical signs and symptoms of irritation (Lorimer et al., 1978) in 488 styrene-exposed workers, the workers were divided into 2 groups, based on estimated exposures of 4.2 mg/m^3 (1 ppm) and > 21 mg/m^3 (> 5 ppm), mostly above 84 mg/m^3 (20 ppm). When asked about lower respiratory symptoms, significantly more workers in the high-dose group responded in the affirmative. There were no differences in length of employment and the prevalence of upper respiratory symptoms between the 2 groups.

In a symptom survey (Härkönen, 1977) of 98 male laminating workers occupationally exposed to styrene, symptoms that fulfilled the British Medical Research Council's criteria for simple chronic bronchitis were more common in the exposed than in an unexposed group. In the exposed group, 28% had simple chronic bronchitis, compared with 12% in the control group ($P < 0.05$). The smoking habits of the groups did not differ.

7.2.8 Endocrine organs

In a study that involved 25 rubber workers, exposed to styrene, and 26 age-matched controls, Wink (1972) did not observe any significant differences in the urinary excretion of 17-ketosteroids or 17-ketogenic steroids. Exposure data were not given. However, Chmielewski et al. (1973) reported reduced excretion of 17-ketosteroids in the urine in 27 out of 67 workers exposed to styrene concentrations of 100-300 mg/m^3 (24-72 ppm), but these findings were not convincingly documented. The evaluation of possible effects of styrene on the steroid metabolism should be studied using more refined techniques.

In the study of Chmielewski et al. (1973), glucose tolerance was observed to be somewhat higher in the exposed group than in the control group, and even more in the group with higher mandelic acid concentrations in the urine (limit 400 mg/litre). Evidence of effects of styrene on the endocrine glands was lacking.

7.2.9 Carcinogenic effects

(a) Styrene production and polymerization processes

A survey of 560 individuals from a styrene production and polymerization plant was reported by Lilis & Nicholson (1976) and Nicholson et al. (1978). In 1960, the workers had been employed in the plant for at least 5 years. It is known that benzene, coke-oven products, butadiene, and ethyl-benzene had been used in this plant prior to 1965. Among 83 deaths, 17 were due to cancer, including 2 cases of leukaemia and one of

a lymphoma. When the total cancer mortality in this cohort was compared with that in the general population, no excess of cancer incidence was found, but the follow-up period may have been too short to detect an increased risk of neoplasia. One case of leukaemia and one of lymphoma were found in 21 additional death certificates of individuals who had been employed for less than 5 years in 1960. In an additional analysis of 444 death certificates on all individuals who had been employed for at least 6 months in the plant, 7 cases of leukaemia and 5 cancers of the lymphatic system were found. However, the populations at risk are not known.

A cohort of 1960 workers employed for at least 1 month in a styrene-polystyrene manufacturing plant was followed from 1931 to 1976 (Frentzel-Beyme et al., 1978). Analysis of deaths occurring (after a minimum period of 5 years) in groups exposed for 5, 10, 15, and over 15 years did not indicate an increase in cancer mortality in any of the groups compared with the reference population. The Task Group noted, however, that the mortality rates for the reference population were only available for the period 1970-75, and that follow-up was incomplete.

Ott et al. (1980) carried out a retrospective cohort study in workers involved in the production of styrene and polystyrene. Mortality experience was followed from 1935 to 1975. Among 282 deaths, 6 cancers of the lymphatic and haematopoietic tissues (except leukaemia) and 6 cases of lymphocytic leukaemia were observed, compared with 4.5 and 2.9, respectively, expected on the basis of US white male mortality rates and 2.6 and 1.6 deaths expected from a comparison industrial cohort. Four deaths from leukaemia were observed in a sub-group of workers exposed to styrene, ethyl benzene, oligomers of styrene, mineral oil, polymer dusts, and extrusion fumes; 0.5 deaths would have been expected on the basis of US white male mortality experience. Only one of the 4 individuals had spent at least 5 years in the working environments under study. Inclusion of cases of leukaemia still alive at the end of the study period or identified through other conditions listed on the death certificate increased the total number of lymphoreticular malignancies to 21 cases. These included 7 lymphocytic leukaemias, 4 other leukaemias, 4 multiple myelomas, 4 Hodgkin's diseases, and 2 other lymphomas. Five of the subjects with lymphocytic leukaemia had worked in the area of polymer extrusion and for 4 of them the disease developed 20 or more years after the first exposure. According to the authors, there was no increase in the general cancer incidence. The incidence of lymphatic leukaemia was, however, more than that expected.

(b) Lamination and related processes in boat building

An epidemiological study was carried out on 1500 workers from 36 companies in the reinforced plastics and plastic boat industry. The majority of the workers were between 30 and 40 years old. The average period of employment was from 6 to 7 years but half of the workers had been in this type of work for less than 3 years, while 54 had been employed for over 20 years. Exposure was estimated to be to an average styrene concentration of 1050-1470 mg/m^3 (250-350 ppm) at the end of the sixties and the beginning of the seventies. Seventeen cases of cancer were found in this group. In 3 of these, the cancer was present before their employment. In the remaining 14 cases, the sites and distribution of the various types of tumours were in accordance with data from the cancer registry. Among the deaths, one case of neoplasm of the lymphatic and haematopoietic system (plasmocytoma) was identified. However, because of the short follow-up period and the small number of deaths it was not possible for the authors to calculate any observed/expected figures (Ahlmark, 1978).

A study is in progress (Tola et al., 1981) in which the tumour incidence in a cohort of 2209 workers, employed in the manufacture of reinforced plastics in 160 workplaces, is being investigated. Exposure to styrene vapour commenced in 1960 and the level of exposure has been estimated to range between 126 and 1260 mg/m^3 (30 and 300 ppm). The majority of workers have been exposed since 1967. Among 27 deaths, 6 deaths from cancer were observed in this cohort (versus 8.1 expected) and the cancers were found in tissues other than lymphatic or bone-marrow tissue. Five of the cancers appeared in workers with 5 or fewer years of exposure. The Task Group noted the low mortality rate.

(c) Styrene-butadiene rubber manufacture

McMichael et al. (1976) analysed the mortality experience during the period 1940-60 in a sample of workers (exact number not specified) taken from a population of 1482 workers in the rubber industry. The subgroup of workers was involved in the manufacture of styrene-butadiene rubber. For malignancies of the lymphatic and haematopoietic systems, a relative risk ratio of 6.2 was observed compared with other workers. Subsequently, Smith & Ellis (1977) reported that this excess was based on 4 cases. A case control analysis of the same data was performed by Spirtas et al. (1976) who calculated a relative risk ratio of 2.4 for lymphatic and haematopoietic neoplasms among these styrene-butadiene workers. Past exposure to solvents and other monomers was suspected.

The mortality experience of 2 cohorts of rubber workers was analysed by Taulbee et al. (1976) using both cohort and case-control analysis. No significant excess of lymphatic and haematopoietic neoplasms was observed.

Case reports of leukaemias and lymphomas among styrene-butadiene rubber workers were reported by Block (1976), Lemen & Young (1976), and Meinhardt et al. (1978).

7.2.9.1 Summary and conclusions

There have been anecdotal reports of a small number of cases of leukaemia and lymphoma in workers employed in the manufacture of styrene-butadiene rubber, but the study populations have been ill-defined and exposure to solvents and other monomers is known to have occurred.

An association between leukaemia and exposure to styrene in the production and polymerization process industries has been suggested in another study. However, the cases of leukaemia occurred in a group with a concomitant past history of exposure to colourants, polymer fumes and, possibly, benzene.

The effects of long-term exposure to styrene are under investigation in at least 2 epidemiological studies on workers employed in the industries involving styrene lamination of glass fibre materials and related activities.

It was the opinion of the Task Group that the data necessary to form an evaluation were inadequate and that a causal relationship between exposure to styrene and human cancer could not be demonstrated at present.

7.2.10 Genetic effects in somatic cells

7.2.10.1 Structural chromosome aberrations

Several studies have been published on structural chromosome aberrations in the peripheral lymphocytes of workers employed in the reinforced plastics industry or in the production of styrene and polystyrene (Table 14). In these studies, exposures have been estimated by measuring styrene air concentrations in the workplace or by determining concentrations of styrene metabolites, in most cases mandelic acid, in the urine of the workers.

In Table 14, the studies are listed according to the industrial process. Positive results have been obtained among workers employed in polyester processing. Nine studies included detailed data on chromosome aberrations in individuals. One report by Watanabe et al. (1981) was considered inconclusive because of the low number (50 or less)

of metaphases analysed per person. The other 8 studies with detailed information were used for the final evaluation. Three studies were reported only in abstract or review articles (Sorsa et al., 1979; Sram, 1981; Vainio et al., 1982; Brøgger, 1982).

The study of Meretoja et al. (1978a) was an extension of an earlier study (Meretoja et al., 1977) and included additional analyses of samples taken from the same workers a year later, after continuous workplace exposure.

One of the abstracts was concerned with a study on children exposed during the fetal period, while their mothers were working in hand lamination of polyester resin.

The only available study on chromosome aberrations among workers employed in the production of styrene or polystyrene was negative (Fleig & Thiess, 1978).

7.2.10.2 Other indicators of genetic damage

Seven studies were available on SCE induction in the lymphocytes of styrene-exposed workers employed in polyester processing (Table 14). Two of the studies were reported only in abstracts or reviews (Sorsa et al., 1979; Brøgger, 1982; Vainio et al., 1982). A slight increase in SCEs was reported in 2 studies (Andersson et al., 1980; Camurri et al., 1982).

In 2 of the studies, peripheral lymphocytes of polyester processing workers were analysed for micronuclei. Meretoja et al. (1978a) and Meretoja & Vainio (1979) reported an increase in micronuclei and "cells connected with a nuclear bridge" in the cultured lymphocytes of 10 workers in polyester plastic product plants; the study included 5 controls (Table 14). Högstedt & Mitelman (1983) found that the frequency of micronuclei increased in 38 workers exposed for 1-23 years to styrene (mean of time-weighted average concentrations in breathing zone 55 mg/m^3 or 13 ppm, range 4-168 mg/m^3 or 1-40 ppm) compared with 20 controls.

Pero et al. (1982) did not find any increase in unscheduled DNA synthesis (UDS) in isolated lymphocytes of 38 workers in a fibreglass-reinforced polyester plastic factory compared with 20 unexposed controls. Styrene air concentrations in the workroom varied between 4 and 168 mg/m^3 (1 and 40 ppm).

In an evaluation of the incidence of malformed children and miscarriages among spouses of men exposed to styrene in a reinforced-plastic boat factory, Andersson et al. (1980) concluded that the number of pregnancies (39 exposed and 41 controls) was too small to reveal mutagenic effects.

Table 14. Summary on studies of structural chromosome aberrations and sister chromatid exchanges (SCEs) in the lymphocytes of workers exposed to styrene.

Industry branch / Reference	No. persons studied		Measured concentration styrene in air (mg/m³)		Years of exposure		Urinary mandelic acid (mg/g of creatinine)		Cells with chromosome aberrations, gaps included (%)[a]			SCEs/cell		
	Exposed	Controls	Average[a]	Range	Mean[a]	Range	Mean[a]	Range	Exposed	Controls	Result[b]	Exposed	Controls	Result[b]
Styrene manufacture														
Flieg & Thiess (1978)	5	20	~2[c]	0-29[c]	21.6	14-25	~30[d]	19-40[d]	3.8	5.5	-	..	..	..
Polystyrene manufacture														
Flieg & Thiess (1978); Thiess & Flieg (1978)	12	20 (12)[e]	~8[c]	0-197[c]	20.3	3-39	~32[d]	<5-100[d]	5.1	5.5 (5.5)[e]	-	.. ..	.. ..	.. ..
Polyester processing														
Meretoja et al. (1977); Meretoja & Vainio (1979)	10	5	..	..	3.2	0.6-8.5	721	23-3257	16.6	1.8	+	..	..	..
Meretoja et al. (1978b)	16	6	..	up to 1260	6.3	1-15	570	23-3257	15.1	2.0	+	..	..	..
(a year later)	11	3[f]	..	up to 1260	8.1	2-16	329	52-1646	16.2	..	+	5.3	4.4	-
Flieg & Thiess (1978)	14	20	~477[c]	<210-1260[c]	7.9	2-24	~593	42->1500	9.2	5.5	+	..	..	..
Andersson et al. (1980)	36	37	~197	0-1008	5.0	0.3-12	..	..	12.3	6.7	+	9.7	8.7	+
	20[f]	20[f]												
Watanabe et al. (1981)	16	13	151-<294	0-886	~4.5	0.6-9.3	~594	90-2640	2.9	3.2	(-)[g]	13.5	13.9	-

Table 14. (contd)

Watanabe et al. (1983)	18	6	168-210	<5-1075	9.7	<1-30	332	1-~1040	6.5	4.7	±	8.9	8.5	-
Thiess et al. (1980, 1982)	24	24	25-244	3-748	~14.4	4-27	..	0-320[c]	5.1	3.8	+[h]	..	..	..
Camurri et al. (1982)[k]	24 (22)[f]	21 (20)[f]	..	30->400	9.4	1-22	472[c]	45-1108[c]	~35.1[i]	~8.4[i]	+	13.9	10.8	+
Abstracts														
Šram (1981)	36	19	..	..	..	..	..	..	1.4[i]	1.3	-	..	..	..
(cited data	(34)[j]								(1.4)[ij]					
of Pchlova & Šram)	22	22	..	..	..	..	..	..	1.7[i]	1.4[i]	-	..	..	..
Brøgger (1982)[k]	13 (8)[f]	0	129	15-364	9.8	1-24	527	292-688	6.4	..	(-)[g]	6.6	..	(-)[g]
Sorsa et al. (1979);	6	10	..	..	..	2-7	..	..	8.8	3.0	+	7.5	8.5	-
Vainio et al. (1982) (in utero exposed children)	7	10	..	..	..	(5-8 months)	..	..	4.9	1.6	+	4.9	5.2	-
Högstedt et al. (1979)	6	6	~162	60-800	4.0	0.5-10	490	225-2100	10.2	4.9	+	..	..	..

[a] Estimated from the data given if not indicated in the report.
[b] Compared to response in control persons: + increase; - no effect.
[c] From Thiess & Friedheim (1978).
[d] mg/litre urine.
[e] Controls in Thiess & Fleig (1978).
[f] For the analysis of sister chromatid exchanges only.
[g] Inconclusive because of the low number of cells analysed (Watanabe et al., 1981) or lack of controls (Brøgger, 1982).
[h] Negative if gaps are excluded.
[i] Gaps excluded.
[j] 6 months later.
[k] Details, not mentioned in the report, were obtained from the author(s).

7.2.10.3 Conclusions

The available evidence suggests that styrene exposure in the reinforced plastics industry with its more intensive exposures is associated with an increased frequency of structural chromosomal abnormalities.

7.3 Effects on Reproductive Function and Teratogenic Effects

In a case-reference study on 63 pairs of mothers from a register of congenital malformations (Holmberg, 1977), 2 mothers with children having central nervous system defects had been employed in the reinforced plastics industry during pregnancy. A third case-mother was also found, who had been exposed to styrene at home.

In a study on spontaneous abortions registered in hospitals (Hemminki et al., 1980a) during the period 1973-76, the following numbers and rates of abortion were found:

- General population 15 482 (5.52%);
- Union of Chemical Workers 52 (8.54%);
- Plastics industry 21 (8.94%);
- Styrene production and use 6 (15%).

The abortion rates for the occupational groups were statistically different from the controls. The abortion rates increased with age in the general population, while they were found to decrease with age among the Union of Chemical Workers. No data were given concerning the relationship with age of spontaneous abortions among women employed in the plastics industry or in styrene production and use; thus the significance of the increased abortion rates among these groups cannot be assessed.

In a small-scale study, Härkönen & Holmberg (1982) recently interviewed 67 female lamination workers of child-bearing age and a similar number of textile and food production workers who were thought to be proper controls. The groups were reported not to differ either in their menstrual behaviour or in the number of spontaneous abortions. The number of deliveries was found to be lower among the styrene workers, which may be partially explained by the higher number of induced abortions in the styrene-exposed group.

8. EXPOSURE-EFFECT/EXPOSURE-RESPONSE RELATIONSHIPS, AND EVALUATION OF HEALTH EFFECTS

8.1 Data from Experimental Animal Studies

8.1.1 Metabolic pathways and kinetics

Pulmonary uptake in man is of greatest importance though uptake through the skin occurs. Styrene is biotransformed largely via the 7,8-epoxide by the mixed function oxidase system.

The kinetic data show that exposure of experimental animals to increasing levels of styrene results in progressive saturation of the metabolic pathways. The half-time of styrene disappearance from the body varies with the dose. The range of exposures tested was from 189 mg/m^3 (45 ppm) to 10 500 mg/m^3 (2500 ppm). The consequence of such metabolic saturation, which has been reported to occur at about 2520 mg/m^3 (600 ppm) in air, is an increase in the proportional deposition of styrene in fat. In human beings the half-time for the elimination of styrene from adipose tissue is 2-3 days.

The principal urinary metabolites are mandelic and phenylglyoxylic acids. Recent evidence suggests that the pattern of urinary metabolite excretion varies with mammalian species.

8.1.2 General toxicity

8.1.2.1 Acute toxicity

A mean odour detection threshold for styrene of 3.06 mg/m^3 (0.73 ppm) was found for unadapted subjects. The odour was reported to be strong but not objectionable at about 420 mg/m^3 (100 ppm). With short-term exposures at concentrations exceeding 840 mg/m^3 (200 ppm), styrene vapour was irritating to the eyes and nose.

The central nervous system effects begin to appear in the range of 210-840 mg/m^3 (50-200 ppm) and distinct impairment of reaction time and body balance were found at concentrations exceeding 840 mg/m^3 (200 ppm).

The acute toxicity of styrene in animals is rather low: the LD_{50} in rats has been reported to be 5 g/kg body weight after oral administration and 2-3 g/kg body weight after intraperitoneal injection. Exposure of rats to styrene vapour at 1300 to 10 000 ppm for up to 4 h caused nasal mucous membrane and eye irritation as well as acute depression of the central nervous system. Marked pulmonary lesions were also noted.

There were no kidney changes in animals exposed to 5460 mg/m^3 (1300 ppm) after a single exposure. Severe irritation

of the eyes and nose was observed in rats and guinea-pigs after exposure to concentrations of 2730-5460 mg/m³ (650-1300 ppm) and general weakness and unsteadiness gradually developed after 12 h.

Acute toxic effects were observed in mice exposed to a styrene level of 21 000 mg/m³ (5000 ppm) for 2 h and guinea-pigs exposed to 84 920 mg/m³ (2600 ppm) for 8 h. A short-term exposure to 1260 mg/m³ (300 ppm) in the air caused only very slight behavioural effects in rats, even though pronounced neurochemical changes were observed.

The high exposure levels used in studies on experimental animals have not been observed in the human environment.

8.1.2.2 Subacute and chronic toxicity

Inhalation of styrene vapour by rats at a concentration of 5460 mg/m³ (1300 ppm) for 7 h/day over several months did not have any deleterious effects on the kidney and did not cause any changes in the erythrocyte count, haemoglobin concentration, and leukocyte count. There were not any essential morphological changes in the rat lungs. Similar results were obtained with rabbits, monkeys, and guinea-pigs except that 10% of the guinea-pigs died after the first exposure with signs of lung irritation. An exposure level of 2730 mg/m³ (650 ppm), for 7 h/day for 214-360 days, appeared not to have any effects on the lungs of guinea-pigs.

Rats exposed by inhalation to a styrene concentration of 1260 mg/m³ (300 ppm) (6 h/day, 5 days a week) for up to 11 weeks developed axonal protein changes in the brain, an induction of drug metabolizing enzymes in the kidney and the liver, histological alterations in the liver, and a depletion of the glutathione (GSH) contents of the kidney and the liver. No significant depletion of GSH occurred at 420 mg/m³ (100 ppm).

Oral subacute studies were carried out on rats and beagle dogs. The rat study was carried out on females and exposure induced only weight increases in the liver and kidney. No effect was observed at a dose of 133 mg/kg body weight. The study on dogs lasted more than 19 months and showed a mild haemolytic anaemia characterized by Heinz body production. Effects were observed down to the lowest dose tested (200 mg/kg body weight).

8.1.3 Genetic effects

Styrene is a potential mutagen only after metabolic activation. Mutagenicity tests in bacteria, yeasts and mammalian cells *in vitro* have yielded contradictory results for styrene that may be due to differences in the efficiency

of activation and inactivation of styrene in different studies.

Conclusive dose-effect relationships could only be established in several in vitro studies.

The results of tests for chromosomal damage induced by styrene in experimental mammals have been contradictory. Three positive studies have been reported; 2 in mice, and 1 in rats. A dose-effect relationship was only observed for the induction of SCE in mice exposed to styrene in air levels of more than 1260 mg/m^3 (300 ppm). Five other animal studies have given negative results. The different end-points, routes of exposure, durations of treatment, species and strains of animals used, and the small number of mammalian studies available, make it impossible to draw definitive conclusions concerning dose-effect relationships.

8.1.4 Carcinogenic effects

Oral administration of styrene to mice induced a significant increase in pulmonary tumours in the O_{20} strain at a dose of 1350 mg/kg body weight and a doubtful increase in B6C3F1 mice at 300 mg/kg body weight. No significant increase in tumour incidence was observed in the $C_{57}B1$ strain, when styrene was administered pre- and post-natally at 300 mg/kg body weight.

In 2 studies on rats, styrene given orally at doses ranging from 500 to 1350 mg/kg body weight did not induce a significant increase in tumour incidence. A greater incidence of lymphomas, observed in an inhalation study on rats exposed to a styrene concentration of 600 mg/m^3 (1000 ppm), could not be causally related to styrene.

Thus, the evidence available indicates that styrene at the doses administered (1350 mg/kg body weight for O_{20} strain) caused an increase in pulmonary tumours in mice.

Styrene 7,8-oxide induced squamous cell carcinomas in the forestomach of rats given doses of 50 and 250 mg/kg body weight. In a second study on rats, both squamous cell carcinomas and pulmonary carcinomas were induced, when styrene oxide was given at a dose of 100-150 mg/kg body weight per day, 4 - 5 days a week.

When styrene oxide was painted on the skin of mice at concentrations of 50 or 100 g/kg, no tumours of the skin were induced.

8.2 Human Studies

8.2.1 Effects on organs and systems

Effects on the following organ systems were investigated: nervous, haematopoietic and immune systems, the kidney and

urinary tract, the gastrointestinal tract, liver, cardiovascular and respiratory systems, and the endocrine organs.

Most studies of the haematopoietic system did not produce any positive data; in one study, a slight decrease in thrombocyte count in workers exposed to styrene for more than ten years was detected.

A clear-cut trend towards altered liver function has not been demonstrated. At low exposure concentrations, the commonly used parameters, such as activity of serum enzymes of hepatic origin, except for γ-GTP, have given equivocal results.

Slight effects on the lower respiratory system were noted in some studies.

No adequate data were available to the Task Group to establish dose-effect or dose-response relationships for the aforementioned systems.

Slight disturbances of visuomotor accuracy and psychomotor performance were noted at styrene levels exceeding 210 mg/m³ (50 ppm), and an increased incidence of abnormalities in electroencephalograph recordings was detected at styrene concentrations below 420 mg/m³ (100 ppm); relationships between the exposure levels and the severity of these effects and the response rates were observed.

While the reaction times were shown to be prolonged for exposure levels below 630 mg/m³ (150 ppm), dose-response relationships could not be established.

8.2.2 Genetic effects in somatic cells

Knowledge concerning the possible genetic effects of styrene exposure on man is still inadequate for any dose-response extrapolations. Available information is limited to structural somatic chromosome damage in the peripheral blood lymphocytes of workers occupationally exposed to high concentrations of styrene in the reinforced plastics industry.

Data on individual exposure profiles are necessary for meaningful interpretation of the results of somatic cell analysis. Furthermore, the role of high occasional peak exposures and low continuous workplace exposures in the induction of chromosome damage in lymphocytes in styrene-exposed workers is not clear. Considering the survival time of the target lymphocytes, exposure data should be available for a period of at least 2-3 years preceding chromosome analysis.

Based on available published data, the following conclusions can be made:

1. No clear dose-response pattern can be recognized. Positive results of chromosome aberrations in styrene-exposed workers were restricted to the reinforced plastics industry, where styrene concentrations in air were high. Negative results have been obtained in the manufacture of styrene or polystyrene where exposures to styrene are lower.

2. Variability in the chromosome aberration frequencies reported in different studies is great. This suggests that several factors such as exposure conditions, the method, and the analytical criteria selected, influence the results.

3. Individual differences are great and may reflect variations in exposure or differences in susceptibility.

The Task Group concluded that the health significance of structural chromosomal aberrations in the somatic cells of persons exposed to styrene could not be assessed at present, but that such effects were undesirable.

8.2.3 Carcinogenic effects

Several case reports and epidemiological investigations have implied an increased risk of lymphatic and haematopoietic system cancer in workers involved in the application of styrene, polystyrene, and styrene-butadiene rubber. However, at present, there is not sufficient evidence to establish a direct cause and effect relationship between styrene exposure and cancer in human beings. Assessment of data has frequently been complicated by concomitant exposure to other volatile substances.

The Task Group concluded that, to date, a causal relationship between styrene exposure and cancer could not be established in man.

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