Special Report

No 9

Styrene Criteria Document

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European Centre for Ecotoxicology
and Toxicology of Chemicals

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PREFACE

This report contains an original review and assessment of the toxicological data for styrene and a quantitative risk assessment (chapters 7 to 9) to provide a scientific basis for setting an exposure limit for styrene (chapter 11). Information on occurrence, production and use, exposure and uptake, and measurement techniques (chapters 3 to 6) has been drawn largely from existing reviews.

The report has been prepared for the European Commission Directorate General V responsible for health and safety at the workplace to assist the Group of Scientific Experts (SEG) in its work to provide advice on the basis of available scientific data. This work is being done under Council Directive 80/1107/EEC, as amended with Council Directive 88/642/EEC, on the protection of workers from the risks related to exposure to chemical, physical and biological agents at work. In drafting this report existing guidance was followed closely (EC Commission publ. EUR 13776- 1992).

STYRENE CRITERIA DOCUMENT

CONTENTS

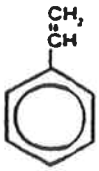
Table of Abbreviations

AAP	Alanine Amino Peptidase
ABS	Acrylonitrile Butadiene Styrene Copolymer
ACGIH	American Conference of Governmental Industrial Hygienists
ALAT	Alanine Amino Transferase
Alb	Albumin
AM	Alveolar Macrophages
AP	Alkaline Phosphatase
ASAT	Aspartate Amino Transferase
AUC	Area Under blood concentration Curve
BAT	Biological Tolerance Value
BEI	Biological Exposure Index
BM	Bone Marrow
CBI	Covalent Binding Index
CEC	Council of European Community
CNS	Central Nervous System
EDI	Estimated Daily Intake
EDTA	Ethylene Diamine Tetraacetic Acid
EEG	Electroencephalography
EHA	Environmental Health Associates
EPS	Expandable Polystyrene
FDA	Food and Drug Administration (USA)
FID	Flame Ionisation Detector
GAL	β-Galactosidase
GLU	β-Glucoronidase
GPPS	General Purpose Polystyrene
GPRMC	European Org. of Reinforced Plastics/Composite Materials
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GSH	Glutathione
GT	Glutamyl Transpeptidase
Hgb	Haemoglobin
HIPS	High Impact Polystyrene
HPLC	High Performance Liquid Chromatography
LDH	Lactate Dehydrogenase

LHC	Lymphatic and Haematopoeietic Cancer
LPG	Liquefied Petroleum Gas
MA	Mandelic Acid
MFO	Mixed Function Oxidase
MN	Micro Nucleus
MS	Mass Spectrometry
NAG	N-Acetyl-β-D-Glucosamidase
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health (USA)
NPSH	Non-Protein Sulfhydryl groups
OSHA	Occupational Safety and Health Administration (USA)
PB-PK	Physiologically-Based Pharmacokinetic
PBL	Peripheral Blood Lymphocytes
PGA	Phenyglyoxylic Acid
PMCC	Polystyrene Manufacturing Colouring and Compounding Plant
RBP	Retinol Binding Protein
RCC	Renal Cell Carcinoma
RD_{50}	50% Decrease of Respiratory Rate
RL	Regenerating Liver
SAN	Styrene Acrylonitrile
SB	Styrene Butadiene Copolymer
SBR	Styrene Butadiene Rubber
SCE	Sister Chromatid Exchange
SDH	Sorbitol Dehydrogenase
SIRC	Styrene Information and Research Center (USA)
SMR	Standard Mortality Ratio
SO	Styrene-7,8-epoxide (Phenyloxirane)
SPI	Society of Plastics Industry
SSB	Single Strand Breaks
STEL	Short Term Exposure Limit
TEAM	Total Exposure Assessment and Management
TWA	Time-Weighted Average
UPR	Unsaturated Polyester Resins
USEPA	Environmental Protection Agency (USA)

1. SUBSTANCE IDENTIFICATION

1.1 IDENTITY

Common name:	Styrene
CAS registry No:	100-42-5
EEC labelling:	R: 10-20-36/38 S: 23
IUPAC name:	Ethenylbenzene
EINECS No:	2 028 515
Synonyms and Styrene trade names:	Vinylbenzene Ethenylbenzene Phenylethylene Phenylethene Cinnamene
Chemical group:	Unsaturated hydrocarbon
Formula:	C_8H_8
Structure:	
Molecular mass:	104.15
Purity of technical product	up to 99.9 %

1.2 STYRENE IMPURITIES

Styrene is one of the purest raw materials in the petrochemical technical industry, typically containing a total of less than 2,500 ppm of impurities.

Potential impurities include:

Benzene	<1 ppm
Cumene	10-350 ppm
vinylbenzene	<10 ppm
Ethyltoluene	10-1,000 ppm
Ethylvinylbenzene	<10 ppm
Methylstyrene	50-1,000 ppm
Phenylacetylene	10-100 ppm
Propylbenzene	10-150 ppm
Toluene	<1 ppm
Vinyltoluene	<50 ppm
Xylene	20-1,000 ppm

1.3 NECESSARY ADDITIVE

4-*tert*-Butylcatechol	10-100 ppm

2. CHEMICAL AND PHYSICAL PROPERTIES

2.1 PROPERTIES

Styrene is a colourless, highly volatile liquid with a characteristic pungent smell. It polymerises easily at room temperature in the presence of oxygen and oxidises on exposure to light or air.

Table 1: Chemical and Physical Properties

Parameter	Value	Reference
Boiling temperature	145.2°C	WHO (1983)
Freezing point	-30.63°C	WHO (1983)
Flash point	31°C	WHO (1983)
Vapour pressure at 20°C	5 mmHg	U.S. Dept Health and Human Services
Colour	Colourless to yellow	U.S. Dept Health and Human Services
Threshold odour concentration	0.02 ppm	Hellman and Small (1974)
Solubility in water	30mg/100ml at 20°C	WHO (1983)
Solubility in organic solvents	ethanol, diethylether, acetone, benzene and petroleum ether	WHO (1983)
Partition coefficient at 20°C	2.95	U.S. Dept Health and Human Services
Flash point	31°C	WHO (1983)
Explosion limit	1.1-6.1%	U.S. Dept Health and Human Services
Auto-flammability, ignition temperature	490°C	WHO (1983)

The technical product is shipped as a liquid. A stabiliser, 4-*tert*-Butylcatechol is added to the styrene which works in conjunction with oxygen to prevent polymerisation.

2.2 CONVERSION FACTOR

Conversion factors for styrene concentrations in air, calculated at 20°C and 1,013 hPa are:

$1 mg/m^3 = 0.235$ ppm
1 ppm $= 4.26 mg/m^3$

3. OCCURRENCE

3.1 ENVIRONMENTAL

Styrene has been identified in both automobile exhaust (Warner-Selph and De Vita, 1989) and cigarette smoke (Byrd *et al*, 1990). Studies on automobile exhaust suggest that motor vehicles are a primary source of man-made emissions of hydrocarbon gases (National Academy of Sciences, 1976), with Guicherit and Schulting (1985) suggesting that approximately 57% of the styrene present in ambient air is attributable to "mobile" sources. Cigarette smoke probably play a minor role in contributing to styrene levels in outdoor air; it may play a more significant role in contributing to indoor exposures. (For more information see 5.3.1 and 5.3.2).

3.2 WORKPLACE

Emissions of styrene into the environment occurs during monomer production and polymerisation processes. During styrene production fugitive emissions have been estimated to be approximately 20 metric tons for every 260,000 metric tons produced i.e. a loss of approximately 0.0076%. With newer plants the levels of emission have been reduced dramatically with only about 3.5 metric tons of styrene emission occurring per 450,000 tons of styrene produced i.e. an emission 0.0007%. About 33g of styrene emissions can occur for each metric ton of polystyrene produced i.e. a loss about 0.0033%. With increasing upgrading of plant and equipment it can be expected that fugitive styrene emissions will continue to decline.

3.3 FOOD

Styrene has been found in a variety of foods and beverages (van den Berg *et al*, 1993). Because styrene is volatile, reactive and rapidly destroyed by ozone and hydroxyl radicals, it is unlikely to be transported to any significant extent, or to be a source of styrene in water or soil (for review see Alexander, 1990). As there is very little possibility of styrene occurring in drinking water or entering the food chain the presence of styrene in food is unlikely to be caused by environmental contamination.

Sources of styrene in food include natural occurrence and/or migration from polystyrene food packaging. An extensive literature search conducted by the Netherlands Central Institute for Nutrition and Food Research (1983) described the presence of styrene in various foods including: apples, various wild berries (bilberry, blueberry, cloudberry, cowberry, cranberry and loganberry), currants, grapes, peaches, strawberries, onions (roasted), capsicum species, peas, tomatoes, vinegar, parsley, cheese, milk, milk products, eggs, fish, beef, beer, rum, whiskey, cider, white wine, cocoa, coffee, tea, filberts, peanuts, peanuts, honey, olives, trassi, walnuts, Brazil nuts, oats and soursop. In many instances no quantitative data were supplied and there is always the possibility that the food samples may have come into contact with polystyrene before analysis. Some reports however do provide an estimate of the levels of styrene occurring naturally in foods; for example a study of the volatile constituents found in dried legumes indicated that the concentration of styrene in split peas and lentils was 4 and

5 µg/kg respectively (Lovegren *et al*, 1979) with the level in German beer (Pilsener) being approximately 70 µg/kg. Styrene is also reported to occur extensively in white wine, possibly due to the decarboxylation of cinnamic acid (Tang and Eisenbrand, 1993). The majority of German wines have been found to contain 1 to 3 µg styrene/kg wine (Tang and Eisenbrand, 1993) while Simpson and Miller (1984) reported concentrations of about 1 to 2 µg of styrene per kg of white wine.

Miller *et al* (1993) and van den Berg *et al* (1993) have provided a quantitative estimation of the levels of styrene occurring naturally in a wide variety of foods including meat, fruit and vegetables. In both studies great care was taken to prevent contact with styrene or any type of styrene based polymer. Van den Berg reported that the concentration of styrene in apples, cauliflowers, onions and tomatoes was below 1 µg/kg while levels in black currants and wheat may be as high as 6 µg/kg and 2 µg/kg respectively. Similar levels were reported by Miller *et al* (1993) i.e. levels in beef, coffee beans, peanuts, wheat, oats, strawberries and peaches varying between 0.3 to 8 µg/kg with no styrene being detected in tomatoes, pecans, milk and chicken - detection limit 0.1 µg/kg. The authors did however report exceptionally high levels, range 157 to 39,200 µg/kg, in cinnamon. The results of such studies suggest that styrene may be a natural constituent of many foods being formed in a number of ways including by bacteria and moulds, from carotenoids, from hydrocarbons, from aldehydes, by Maillard reactions, during autoxidation of methyl arachidonate and from 2-phenylethanol (van den Berg *et al*, 1993).

Besides natural occurrence, styrene in food may be due to migration of the monomer from styrene based polymers used for food packaging. (For example see Varner and Breder, 1981; MAFF,1983; Gilbert and Starin, 1983 and Society of Plastics Industry, 1993). Migration is dependent on a number of factors including the residual styrene content in the polymer, the nature of the packaged food (migration into fatty foods being higher than aqueous based materials), the contact area, duration of contact, temperature of both storage and package filling, and a variety of other factors. Fatty foods e.g. butter, oil, margarine, dairy products, which have the highest migration levels show styrene concentrations ranging from <5 to 25 µg/kg (Tang and Eisenbrand, 1993).

4. PRODUCTION AND USE DATA

4.1 PRODUCTION OF STYRENE

The raw materials used to make styrene are obtained from crude oil or liquefied petroleum gas (LPG). A range of processes are required to transform the crude oil or gas into styrene. These processes are described briefly.

The initial step involves a distillation process in which crude oil is refined to produce naphtha heating oil and gasoline. The naphtha fraction from the refinery is subsequently processed by steam cracking into ethylene, propylene and a mixture of monocyclic compounds including benzene. The benzene, after passing through a drying column into a reactor, is mixed with ethylene in the presence of a suitable catalyst. This alkylation reaction is exothermic (the heat being used to generate process steam that can be used in the distillation processes) and can be carried out in liquid phase at low temperatures and pressures with an aluminium chloride catalyst, or in the vapour phase at higher temperatures and pressures with a zeolite catalyst. The crude ethylbenzene is purified by distillation to recover unreacted benzene for recycling. Distillation ends and by-products are used for energy recovery.

The next step in the process is the dehydrogenation of ethylbenzene to produce styrene. More than 90% of styrene produced in the world is made by the iron oxide-catalysed dehydrogenation of ethylbenzene. Continuous chemical reactors are used for this reaction that is carried out at high temperature (typically over 600°C using steam as the energy source) and low pressure. Heat is recovered and the reaction mixture is sent to the fractional distillation units for purification.

The crude styrene from the dehydrogenation reaction is purified in several stages using fractional distillation. The resulting product is commercial polymerisation quality styrene. By-products are recycled or used for their fuel value.

Table 2: Annual Production of Styrene - World Summary

Location	Styrene Capacity (Thousands of Tons)					
	1990	1991	1992	1993	1994**	1995**
US	3,818	4,571	4,853	5,283	5,283	5,283
Canada	825	775	735	735	735	735
Western Europe*	3,780	3,770	4,010	3,940	4,320	4,360
Japan	2,125	2,270	2,320	2,360	2,570	2,880
Latin America	580	580	580	535	670	695
Eastern Europe	1,450	1,370	1,340	1,340	1,425	1,500
Middle East	360	360	360	360	420	420
East Asia/Far East	1,249	1,629	2019	2,094	2,134	2,408
Total capacity	14,187	15,325	16,217	16,647	17,557	18,281
Total Production	13,561	13,903	14,038	14,168	14,879	15,625

* Actual production in Western Europe in 1992 was 3.5 million tons

** Estimated

Information on production obtained from PCI Consultants Ltd., 1992 World Styrene Monomer and Derivatives

4.2 MAJOR USES OF STYRENE

The major use of styrene is in the production of homo- and copolymers (general purpose polystyrene (GPPS), high impact polystyrene (HIPS) and expandable polystyrene (EPS), acrylonitrile-butadiene-styrene (ABS), styrene-acrylonitrile (SAN), styrene-butadiene latex (SB latex), styrene-butadiene rubber (SBR), unsaturated polyester resins (UPR) and a variety of other resins). Information on these applications is provided below.

Table 3: Uses of Styrene - World-wide

Application	Styrene Used (Thousands of Tons)					
	1990	1991	1992	1993	1994*	1995*
GPPS and HIPS	7,193	7,327	7,316	7,560	7,901	8,259
EPS	1,663	1,680	1,702	1,743	1,842	1,950
ABS/SAN	548	1,454	1,713	1,768	1,846	1,968
SB Latex	884	875	909	944	1,014	1,082
SBR	921	855	856	847	874	909
UPR	720	676	649	699	726	756
Others	583	605	647	650	676	701

* Estimated

Table 4: Uses of Styrene - Western Europe

Application	Styrene Used (Thousands of Tons)	
	1991	1992 (estimate)
GPPS and HIPS	1,832	1,813
EPS	633	602
ABS/SAN	349	353
SBR	157	157
SB Latex	332	343
Resins (UPR and Others)	340	343

Information on applications obtained from PCI Consultants Ltd., 1992 World Styrene Monomer and Derivatives.

Polystyrene can be produced by free-radical, anionic, cationic or Ziegler reactions. Of these only the free-radical process is of major commercial interest. Anionic polymerisation is used commercially only for producing block copolymers of styrene with butadiene. Cationic polymerisation is used to produce low molecular weight polystyrene for coatings and glues.

As stated above styrene is in the main polymerised to polystyrene by a free-radical reaction using one of two primary methods i.e. the suspension process or the solution process.

Suspension polymerisation is always run as a batch process. While the major application is in the production of EPS it is also used to produce GPPS and HIPS. In a typical process for GPPS water and styrene are fed to the reaction kettle along with one or more initiators and a suspending agent. The reaction is allowed to proceed at temperatures up to 140°C for about 5-24 h. to almost complete conversion, after which the product is separated, dried and extruded into pellets.

The continuous solution process has evolved as the process of choice for polystyrene manufacture. Reasons for this include that high purity polystyrene i.e. low residual monomer products, can be produced at low cost. For the process there may be from one to five reactors in various configurations. To produce GPPS, the solvent (usually ethylbenzene), and occasionally an initiator are fed to the first reactor. Reactors are usually run at sequentially higher temperatures from 90 to 180° C, with final conversion at 60-90%. The solution is passed to a vacuum devolatilizer that evaporates the remaining monomer and solvent. The hot melt is extruded and pelleted. The monomer and solvent are condensed and recycled to the reactor feed. For the manufacture of HIPS, rubber solution is fed into the first reactor.

5. QUANTITATIVE INFORMATION ON EXPOSURE AND UPTAKE

5.1 OCCUPATIONAL EXPOSURE

5.1.1 Production of Styrene and Styrene Based Polymers

Of the styrene produced in Europe approximately 95% is used in the production of styrene based polymers and latex. The remainder is used in the reinforced plastics and resins industry, as a solvent and reaction intermediate. The available information for the styrene production is provided in Table 5, and for the styrene based polymer production in Table 6. The TWA (8 hrs) measured are below 1 ppm except for SBR. Peaks of exposure are observed particularly during maintenance operations. The levels are very low for ABS Resin production but relatively high for the SBR units. The high level of 50 ppm maintenance is associated with cleaning and washing of vacuum trucks.

Table 5: Styrene Measurements (ppm) - *Monomer Production

Work Area/Process	Exposure Peak (ppm)	Representative 8h-TWA (ppm)
Cracking/Distillation	<1	<1
Purification	<10	0.4
Maintenance	1 to **50	0.1 to 0.5
Laboratory	<5	0.1 to 0.5

* Data supplied by Styrene Sector Group of the European Chemical Industry Council (CEFIC)

** the highest level of 50 ppm is associated with one specific job - washing vacuum trucks (with very low numbers of individuals being exposed).

Table 6: Styrene Measurements (ppm) - *Polymer Production

Work Area/Process	Exposure Peak (ppm)	Representative 8h-TWA (ppm)
ABS	0.01 to 0.8	0.04 to 0.11
GPPS, HIPS and EPS	<20	0.1 to 0.3
SBR	5 to 25	14 to 16

* Data supplied by European Chemical Industry Council (CEFIC)

5.1.2 Processing of Styrene Based Polymers

During polystyrene processing some decomposition may occur resulting in the emission of small amounts of fumes containing products such as hydrocarbons (including styrene) and aerosols. The concentrations of styrene in such fumes depend on following conditions:

1. Polystyrene type
2. Type of processing equipment
3. Processing temperatures and residence time
4. Ventilation of the working area and local ventilation above the die.

For convenience polystyrene processing can be divided into injection moulding and extrusion and while other processes are used these are minor by comparison.

Injection moulding is a process by which resin is melted and injected in a mould cavity. The injected melt is subsequently cooled down solidifying into the shape of the mould. The resulting form is a finished part needing little further work before assembly into or use as a finished article. Rejected parts can be recycled directly.

Applications of injection moulded articles include foodstuff packaging such as salad containers, yoghurt cups and drinking cups. Other applications include office supplies, television housings, audio and video cassettes, compact disc cases, toys, furniture parts and many other uses. Some examples of styrene exposure during injection moulding of polystyrene are shown in Table 7

Table 7: Styrene Measurements (ppm) - Polystyrene Moulding

Work Area/Process	Exposure (ppm)	Reference
Above mould cavity	ND (0.1)	Seymour *et al* (1978)
Above exhaust vents	2 to 6	Bohl (1982)
Operator location	ND (2)	Mattler (1982)
Operator location	ND (0.05)	Mayer *et al* (1983)
Operator location	max approx 2.5*	Twisk (1991)

ND: Not Detectable. Figure in parentheses represents level of detection

* majority of measurements below level of detection i.e. 0.05 ppm
Operations between 225 to 280°C

Extrusion uses the greatest quantity of polystyrene, with the majority going into sheet production. Extrusion is a process in which the pelleted resin is melted and then forced continuously through a die to form an endless profile of polymer. The polymer which is in a thermoelastic state can be thermoformed to a wide range of shapes using a mould cavity, a stamp and/or vacuum.

In the in-line process the sheet (subsequent to the extrusion without additional heating) is thermoformed directly. In the two-step process the sheet is first wound in large rolls. For thermoforming the sheet is unwound from the roll and then heated. In both processes after thermoforming the articles are separated from the surrounding sheet, leaving a trim skeleton which is recycled directly. Examples of styrene exposure during polystyrene extrusion are shown in Table 8.

Table 8: Styrene Measurements (ppm)- Polystyrene Extrusion

Work Area/Process	Exposure (ppm)	Reference
Foam extrusion	<1	European Chemistry Industry Council
Above sheet extruder	5.13	Seymour *et al* (1978)
Operator location	0.03 to 6.92	Bohl (1980)
Above exhaust vents	2 to 6	Bohl (1982)
Operator location at extrusion die	max 0.16	Mattler (1982)
Fumes from extrusion	max approx 1.0*	Twisk (1991)

Figure in parentheses represents level of detection

* majority of measurements below level of detection i.e. 0.05 ppm

Applications of thermoformed parts include disposables such as cups and plates, meat, poultry and produce trays, hinged containers, multiple and single-serving food containers in dairy and delicatessen markets, vending machine cups, trays for hospital and restaurant use.

In the extrusion/thermoforming process the scrap percentage can range from 15 to 50%. All this scrap is recycled without impairing the properties of the finished article significantly.

5.1.3 Reinforced Plastics

The vast majority of studies on the health effects of styrene have been conducted in the reinforced plastics industry. In this industry styrene exposures are usually relatively high because of the production processes employed. Most operations use hand lay-up or spray-up to deposit polyester resin (containing 30-40 percent styrene) onto a reinforcement, usually fibreglass. Spraying or hand brushing and rolling of the resin mix onto the laminate is usually deposited on large surface areas of the manufactured part. Because of the large surface area, the potential for styrene volatilisation is high. It has been estimated that up to ten percent of the styrene contained in the resin mix evaporates into the workroom air. Other styrene manufacturing and using industries, for example the polystyrene industry, operate with closed processes where the opportunity for worker exposure to styrene is less, and the number of workers are fewer.

The reinforced plastics industry is engaged in the manufacturing of many diverse items. The larger the part manufactured, the higher the styrene exposure is likely to be, because application of the ventilation controls in the manufacture of large parts is difficult. Items manufactured include boats, underground storage tanks, above ground storage tanks and silos, bathtubs and shower stalls, fibreglass panels, and small parts of all sizes and shapes. The industry utilises a variety of processes to manufacture these different items.

One of the most common processes is hand lay-up, where polyester resin containing styrene is applied to the laminate, then rolled or "squeezed" to eliminate bubbles and distribute the resin. In this process the resin is often applied by spraying the mix directly onto the laminate surface. Another common process, spray-up, involves the spraying of both the resin containing styrene and chopped fibreglass simultaneously into the mould. These processes generate styrene in the working atmosphere because of either the atomising of the styrene from the spray operation, or evaporation from the surface of the part.

Data concerning the air levels of styrene in the reinforced plastics industry are shown in Table 9. Other information on exposures can be found in Tables 11,12 and 13 which summarise the results of cytogenetic studies in humans which were, in the main, conducted in workers from the reinforced plastics industry. In addition to the tabulated data Astrup Jensen *et al* (1990) reported an analysis of historical styrene concentrations in air in the Danish reinforced plastics industry for the period 1955 to 1988. In summary the data showed that mean styrene exposure measured as 168 ppm from 1955 to 1970 dropped to approximately 65 ppm during 1971 to 1980 and to about 40 ppm during 1981 to 1988. This general trend towards lower mean exposures is also seen in information from other countries (Kogevinas *et al* 1993) and although decreases may have occurred over different periods in each country the overall pattern of reduction is similar to that seen in Denmark. Despite the reduction in mean levels of exposure Austrup Jensen *et al* (1990) still found maximum concentrations exceeding 900 ppm for the period 1981 to 1988 although in most industries the maximum level for this period was much lower e.g. boatyards range 1 to 270 ppm, carriage body works 2 to 73 ppm, building industry 1 to 144 ppm and furniture industry 1 to 178 ppm. These latter figures are in agreement with Sorsa *et al* (1991) who reported TWA 8 h concentration of styrene in personal air to be in the range 5 to 182 ppm and Geuskens *et al* (1992) who found TWA to range from 11 to 140 or 4 to 127 ppm for workers involved in spraying operations or manually applying the resin respectively. These data indicate that air levels of styrene found in the reinforced plastics industry are substantially higher than those encountered in styrene polymerisation processes.

Many other chemicals, in addition to styrene, are utilised in the reinforced plastics industry. In order to utilise the polyester resin containing styrene in the polyester manufacturers must inhibit the polymerisation reaction until it is required by the user. The user then must promote and catalyse the polymerisation reaction to produce his product. The chemicals used for these purposes include methyl ethyl ketone peroxide and other peroxides, and cobalt compounds. Solvents are frequently used in the industry. The polyester resin is quite viscous and tools and spray equipment need frequent cleaning. Solvents such as acetone, toluene, xylene, methylene chloride, and methyl ethyl ketone are used in large quantities.

Fibreglass is used frequently in the reinforced plastics industry and dust can be generated in the spray-up operation and in grinding the finished laminate. Many shops, particularly the boat builders, utilise woodworking chops and wood dust can also be generated.

Table 9: Styrene Measurements (ppm) - Reinforced Plastics Industry

Work Area/Process	Peak Exposures - Unless Indicated (ppm)	Reference
Various	15-100	Bargodej *et al* (1960)
Boat building	24-90	Zielhuis *et al* (1964)
Various	5-195	Simko *et al* (1966)
Various	50-100	Huzl *et al* (1967)
Various	>600	Matsushita *et al* (1968)
Various	20-290	Gotell *et al* (1972)
Various	45-550	Bodnie *et al* (1974)
Boat	20-300	Kallioski *et al* (1976)
Boat	35-110	Rosenteel *et al* (1977)
Boat	10-170	Bergman (1977)
Various	250-350	Ahlmark (1978)
Various	4-291	Engstrom *et al* (1978a)
Various	40-230	Brooks *et al* (1979)
Boat	3-14	Kjellberg *et al* (1979)
Boat	2-180	Crandall (1981)
Various	0 - >300	Schumacher *et al* 1981)
Boat	1-256	Ikeda *et al* (1982)
Various	0-196	Guillemin *et al* (1982)
Various	28-161	Apostoli *et al* (1983)
Boat	20-135	Todd and Schulman (1984)
Boat	31 and 81 (average)	Lemasters *et al* (1985a)
Boat	72 and 100 (average)	Okun *et al* (1985)
Various	40-100	Coggon *et al* (1987)
Various	24-94	Geuskens and van Hemmen (1989)
Various	4-168	Geuskens *et al* (1992)
Various	45 (average)	Pfaffli (1993)
Various	1-180	Gobba *et al* (1993)
Boat (Belgium)	0.5-99 (8 h-TWA 1-46 ppm)	*GPRMC (1994)
Polyester Production	1-100 (8 h TWA 1-8 ppm)	*GPRMC (1994)

* Information supplied by European Organization of Reinforced Plastics/Composite Materials (GPRMC)

5.2 BIOLOGICAL MONITORING

Because of the efficient metabolism of styrene and rapid excretion of the two major metabolites mandelic acid and phenylglyoxylic acid either mandelic acid, phenylglyoxylic acid or the sum of these two metabolites can be used as biomarkers of styrene exposure. Mandelic acid is excreted in larger quantities than phenylglyoxylic acid (Guillemin and Bauer, 1978). After inhalation exposure to styrene, the elimination half-lives measured in volunteers were 3 to 4 h and in occupationally exposed workers between 5 and 10 h (Guillemin and Bauer, 1979; Ikeda *et al*, 1974). The elimination half-life of phenylglyoxylic acid measured in volunteers was about 8 h.

Urine specimens for the determination of mandelic acid should be collected preferably at the end of the shift in plastic containers. Since urinary concentrations are dependent on urine flow, creatinine determination in the same specimen is necessary. Samples should be stored frozen or acidified and stored refrigerated.

Urine specimens for the determination of phenylglyoxylic acid should be collected at the end of the shift. Because of the rapid degradation of phenylglyoxylic acid, samples should be acidified, stored refrigerated, and analysed no later than 2 days after collection (Guillemin and Bauer, 1978).

Total mandelic acid and phenylglyoxylic acid determination in urines collected prior to the next shift are recommended by some authors, especially in plants where airborne styrene exposures vary greatly during the workday (Bartolucci *et al*, 1986).

Normally, using specific analytical methods, mandelic acid or phenylglyoxylic acid are non-detectable in a population not occupationally exposed to styrene or to other chemicals metabolised to these products.

In a series of human volunteer and occupational exposure studies the correlations between airborne styrene concentrations and the concentration of mandelic acid or phenylglyoxylic acid have been assessed (Berode *et al*, 1986; Ramsey *et al* 1980; Guillemin *et al*, 1979; Engstrom *et al*, 1978 a,b and c; Droz and Guillemin 1983). There was a very good agreement among data obtained in all studies. According to the correlation equations, the concentration of mandelic acid in the urine of a person exposed for 8 h at a time-weighted average airborne concentration of 50 ppm (213 mg/m3) equals a value of 800 to 1,000 mg/g creatinine for end-of-shift samples. Phenylglyoxylic acid concentrations are about 200 mg/g creatinine in end-of-shift samples and 100 mg/g creatinine in pre-shift samples. Bartolucci *et al* (1986) found that exposure to 50 ppm styrene (8 h time-weighted average) equals a concentration of the sum of mandelic acid and phenylglyoxylic acid of 410 mg/g creatinine for pre-shift samples.

Urinary concentrations of both mandelic acid and phenylglyoxylic acid can be affected by exposures to other chemicals which are also metabolised to these biotransformation products such as ethylbenzene, methyl-phenyl ketone. Other factors which affect urinary concentrations are exposures to solvents whose metabolism is mediated by the cytochrome

P-450 enzyme system (benzene, xylene, toluene).

Furthermore, the metabolism of styrene can be influenced by the use of alcoholic beverages. Dermal exposure to styrene increases urinary levels of both mandelic acid and phenylglyoxylic acid.

For the biological monitoring of styrene, the determination of mandelic acid in urine collected at the end of shift is recommended, as an indicator of 8 h time weighted average daily exposure to styrene. The 1993-1994 ACGIH Biological Exposure Index (BEI) is 800 mg mandelic acid/g creatinine (0.6 mol/mol) in the end of shift. For pre-shift samples (next morning) the BEI is 300 mg/g creatinine. The German Biological Tolerance Value (BAT) is 2 g/L (end of shift) and the CEC recommends 1 g/g creatinine (post shift) or 330 mg/g creatinine (pre-shift).

Using phenylglyoxylic acid in urine as a biomarker for styrene exposure, the 1993-1994 ACGIH BEI is 240 mg/g creatinine and the CEC recommends 350 mg/g creatinine in end-of-shift samples. For pre-shift samples (next morning) the BEI is 100 mg/g creatinine. From these data it can be derived that the BEI for the sum of mandelic acid and phenylglyoxylic acid would be 400 mg/g creatinine, for next morning samples. Some data on such measurements are included in Tables 11, 12 and 13 (see Chapter 7.3).

5.3 ENVIRONMENTAL EXPOSURE

5.3.1 Ambient Air

A large amount of information exists on the concentrations of styrene in the atmosphere, especially in cities. The concentrations are usually below 1.0 ppb (by volume) but higher values have also been found, and levels of 5.0 ppb or higher in urban air have been recorded. The amounts rise during pollution episodes and are affected by the season of year (being higher in winter than in summer in New Jersey cities, for example) and whether it is night or day (Harkov *et al*, 1984; Hartwell *et al*, 1987a, 1987b). Levels in downtown Los Angeles ranged from 0.1 to 5.5 ppb (Grosjean and Fung, 1984). The geometric means in three cities in New Jersey ranged from 0.07 to 0.24 ppb in 1981 and 1982 (Harkov *et* al, 1984). The available data for atmospheric concentrations in the United States through 1983 have been summarised and evaluated, some locations having no detectable levels of styrene (Brodzinsky and Singh, 1983).

Data are also available from other countries. In Delft, the Netherlands, average styrene levels measured in 1975 were less than 0.1 ppb with maximum values of 0.7 ppb (Bos *et al*, 1978), but further analyses in the Netherlands in 1980 gave mean values for the test areas of 0.02 to 0.35 ppb and maximum concentrations of 0.15 to 6.40 ppb. Styrene may also be emitted to the air around hazardous waste sites. The mean concentrations in the air have been reported to range from 0.11 to 1.53 ppb with a maximum concentration reaching 15.5 ppb. Sanitary landfills receiving municipal and non hazardous industrial wastes have mean concentrations at locations in New Jersey of 0.23 to 0.41 ppb and maximum values of 1.09 to 1.52 ppb. The concentrations vary appreciably on a daily basis (Harkov *et al*, 1984).

While the presence of styrene in ambient air must be in part related to the industrial production of plastic resins and synthetic rubber a major source is automobile exhaust. Chemical characterization of emissions from contemporary production model light-duty gasoline vehicles has shown that styrene is a component of automobile exhaust (Warner-Selph and De Vita, 1989). Using the Federal Test Procedure, (Code of Federal Regulations, Title 40, Chapter 1. Part 86, Subpart B, section applicable to light-duty vehicles), the average styrene emissions from a Ford Taunus or Toyota Camry were 0.16 or 0.33 mg/km respectively. Air measurements of highway tunnels have indicated overall hydrocarbon emission rates of 7.0 and 6.2 mg/km (for gasoline powered vehicles) and 1.4 and 2.1 mg/kg (for diesel trucks) (Hampton *et al*, 1983). Such data are supportive that motor vehicles are a leading source of man-made emissions of hydrocarbon gases (National Academy of Sciences, 1976), and support the findings of Guicherit and Schulting (1985) who suggested that approximately 57% of the styrene present in ambient air could be attributed to "mobile" sources.

Available information on the fate of styrene in air suggests that because of the high degree of reactivity, especially in the presence of ozone and hydroxyl radicals, it is broken down very rapidly - styrene has a lifetime of about 3 h. Because it absorbs little light at the wavelengths of sunlight reaching the earth's surface it is unlikely that direct sunlight is effective in destroying styrene. The dominant atmospheric removal process for styrene is the reaction with hydroxyl radicals to produce formaldehyde and benzaldehyde although in polluted air in cities the ozone concentrations may sometimes be sufficiently high for ozone to destroy styrene more readily than hydroxyl radicals (Grosjean, 1985). As styrene is destroyed rapidly in the atmosphere it is probably not transported to a significant extent in air. Thus it is very unlikely that the presence of styrene in water or soil can be attributed to air emissions from a source located any distance from the contaminated area. (For review see Alexander, 1990).

Using the level of 1.64 µg/m^3 as the "national average" concentrations of styrene present in personal air (air samples collected at various geographical locations within the US; Wallace, 1987, Wallace *et al*, 1987 and Wallace *et al*, 1988) and assuming an individual inhales 20 m^3 of air daily and 66% of the styrene is absorbed via the lung tissue (Ramsey and Young, 1978), the personal daily absorbed dose from atmospheric styrene is approximately 22 µg/person/d. This is very close to the value of 18 µg/person/d, calculated by Guicherit and Schulting (1985) as being the approximate styrene exposure via the air in the Netherlands, and the levels of 23 and 34 µg/person/d estimated exposures for non-smokers and smokers respectively in Germany (Tang and Eisenbrand, 1993). A study on styrene exposure in the Canadian general population (Newhook and Caldwell, 1993) indicated that exposure (ambient and indoor air combined) ranged from 0.074 to 0.27 µg/kilogram bw which, assuming an adult weighs 60 kg, provides an intake varying between 4.5 to 16 µg/person/d.

5.3.2 Indoor Air

An extensive study of indoor air quality in family homes by Shah and Singh (1988), involving 2125 measurements, indicated an average styrene level of 1.41 µg/m^3. The presence of smokers in the household can however affect the level of styrene in indoor air; for example

Wallace and Pellizar (1986) reported that in winter and autumn styrene levels in the homes of smokers were approximately double that found in the homes of non-smokers, i.e. 2.2 versus 1.1 $\mu g/m^3$ for smokers and non-smokers respectively. While the difference between the two groups narrowed in summer and spring, (presumably because of increased ventilation), the styrene levels in the air of homes with smokers were still higher than non-smokers i.e. 1.1 and 0.8 $\mu g/m^3$ respectively.

5.3.3 Soil

Due to its volatile properties soil contamination with styrene is not an issue. In cases of accidental spillage, transport into the soil can occur. At the surface volatilis ation is important with the half life for the volatilisation of styrene being at about 1 minute. Consistent with many other lipophilic chemicals with increasing penetration, volatilisation becomes slower and subsequently the degree of adsorption to soil or solids (the greater the adsorption the slower the transport) and biodegradation become important criteria for determining downward movement (Alexander, 1990).

5.3.4 Water

An extensive amount of monitoring has been conducted in the United States to determine styrene levels in drinking water. In the National Screening Program for Organics in Drinking Water styrene was not found in drinking water obtained from 102 surface water supplies and 12 ground water supplies (Boland, 1981). A survey of 945 municipalities divided into populations of greater than and less than 10,000 people also failed to find a single site containing styrene (Estrick *et al*, 1984). In a study of 1,701 private and community wells in Wisconsin only 1 was found to contain a detectable level (detection limit 0.3 or 0.5 µg/l) of styrene (Krill and Sonzogni, 1986). Similarly, a survey of information from 1970-1985 on drinking water from the Great Lakes demonstrated that most water samples did not contain detectable levels of styrene, or the levels reported were near the detection limit. While there are a few reports describing the presence of low levels of styrene in water (e.g. 0.024 µg/l; for examples see Alexander, 1990) the majority of studies have failed to find any evidence of water contamination. A comprehensive investigation conducted by the US EPA reported that three national surveys and a large number of state and local surveys had failed to find styrene in drinking water. This mass of information led to the conclusion that styrene is not likely to occur in drinking water (US EPA, 1987).

Information from one of Environment Canada data bases indicate that in over 500 well, reservoir and drinking water samples styrene was only found at trace levels (0.5 µ/l in one sample) (CEPA, 1993).

A report on styrene published by the World Health Organisation (1983) also concluded that while there are sporadic episodes of styrene contaminating water, usually through, improper disposal, it appears that styrene is not a frequent contaminant nor is it usually present in large amounts.

5.3.5 Food

The likely intake of styrene from the diet can be estimated using a number of different methods. Since each differs somewhat in the data input required and the types of calculations that are applied a description of the methods and the results obtained are summarised.

A method to obtain an Estimated Daily Intake (EDI) for styrene combines measurements of styrene levels in various food types with consumption and packaging data. Substantial data on the levels of styrene in various food types are available from the published literature (see Eiceman *et al*, 1982; Gilbert and Startin, 1983; Matiella and Hsieh, 1991; Santa Maria *et al*, 1986; Vamer and Breder, 1981 and Withey and Collins, 1978). The information shown in Table 10 is a summary of published data on styrene levels in various food types (published data) together with an estimate of the average daily human consumption (Pao *et al*, 1982). These data show a dietary intake of styrene with a consumption of 1.35 kg of food is 1.87 µg. Assuming these data are representative of the "total" diet and using the FDA recommendation that 8% of the average of 3 kg of food consumed daily is in contact with polystyrene the EDI may be calculated:

3/1.35 x 1.87 µg/kg = 4.15 µg

In addition to the data appearing in published literature the Plastics Institute of Australia has compiled information on the levels of styrene migrating into food for the National Health and Medical Research Council. This information was based on the levels of styrene monomer measured in a variety of food types packed in styrene-based food-contact polymers (Shields, 1980). The mean level of styrene in food packaged in polystyrene was 29 µg/kg. Once again using the recommendation from the FDA that of the 3 kg of food consumed daily only 8% is in contact with polystyrene, the EDI may be calculated:

29 µg/kg x 3 (kg of food consumed per day) x 0.08 (% of food contacting polystyrene)
= 6.96 µg.

In 1983, the Ministry of Agriculture, Fisheries and Food in the UK. published the results of a very thorough survey of the levels of styrene in food-contact materials and in food (MAFF, 1983). The levels of styrene varied depending on the nature of the food, the amount of residual styrene in the container, and the length and temperature of storage. It was also noted that migration was lower into aqueous foods than into fatty foods. Based on the results of this extensive survey, the average and maximum likely per capita intakes of styrene from the average UK diet were estimated to be approximately 1µg/d and 4 µg/d respectively. It was concluded that, in view of the very low exposure to styrene from food as detailed in the report, "there is no likely toxicological hazard to man from present levels of styrene in food".

Table 10: Daily Oral Exposure of Styrene Via Foods Commonly Eaten by the General Public

Food Type	Average Daily Consumption[1] (kg)	Quantity of Food Contacting Polystyrene[3] (kg)	Styrene Content (µg/kg)	Average Daily Styrene Consumption* (µg/person/d)
Coffee	0.40	0.032	15.28	0.49
Tea	0.24	0.019	15.28	0.29
Hot Chocolate	0.09	0.007	13.00	0.01
Fruit "aid"	0.13	0.010	29.70	0.30
Yogurt	0.08	0.006	24.80	0.15
Cream	0.01	0.006	12.60	0.01
Cottage/Soft Cheese	0.04	0.001	6.4	0.02
Hamburger	0.05	0.003	2.5	0.01
Ground Beef	0.04	0.004	3.0	0.01
Chicken Breasts[2]	0.05	0.004	9.5	0.04
Sausages[2]	0.03	0.002	2.7	0.01
Prepared Salad	0.03	0.002	5.0	0.01
Strawberries	0.04	0.003	5.0	0.02
Gelatin/Cream Desserts	0.05	0.004	30.0	0.12
Jello/Jelly	0.01	0.001	270.0	0.27
Eggs	0.06	0.008	14.0	0.11
Total	1.35			1.87

1 Average daily consumption from Human Nutrition Information Service, Home Economics Research Report No. 44.

2 Estimated levels in "cooked meat", i.e. 15% of level found in raw meat, 85% of styrene having assumed to have been lost by volatilization.

3 The food-type distribution factors (ie quantity of food considered to come into contact with polystyrene packing) is 8%. This figure as obtained from the Food and Drug Administration, Recommendations for Chemistry Data for Indirect Food Additive

* Calculated by the task force

Tang and Eisenbrand (1993) also estimated human exposure to styrene from food in Germany. Using a total per capita consumption of 970 kg/year and assuming that all food is packed in polystyrene giving a styrene content of 20 µg/kg they calculated styrene intake from food to be in the order of about 5 µg/d.

The Society of the Plastics Industry, Inc. (SPI, 1993) have completed a study to evaluate the daily intake of styrene resulting from contact of food simulants with styrene-based food-contact polymers including single-service food packaging materials, disposable (single use) food contact articles and repeated-use articles. Using experimentally-derived diffusion

coefficients for styrene monomer from food packaging materials contacting food simulant solvents (Murphy *et al.* 1992), the maximum daily potential styrene migration from the various food-contact uses of styrene-based polymers was calculated to be approximately 9.06 µg/d.

An examination of the available data shows that, despite the differences in the various methods, all of the approaches produced very similar numerical estimates ranging from about 1 to 9 µg/d. An important point is that, while these estimated dietary intake values are very low, they should still be considered "worst case" and as such they probably represent a substantial over-estimate of actual human dietary exposures to styrene.

5.3.6 Lifestyle

As styrene has been detected in the volatile fraction of cigarette smoke it is to be expected that smokers have a higher exposure to styrene than non-smokers. In a study of mainstream cigarette smoke Baggett *et al* (1974) reported 18 µg of styrene per cigarette while Byrd *et al* (1990) reported the presence of 2.1 µg styrene in "reference cigarettes". Tang and Eisenbrand (1993) calculated that cigarettes with a high amount of condensate will produce about 10 µg styrene per cigarette. Assuming 20 cigarettes are smoked per day with about 70% of the styrene being taken up in the lungs the absorbed amount of styrene from smoking could be as high as 140 µg/d. Although smoking may increase, substantially, environmental exposure to styrene it is important to remembered that cigarette smoke contains at least 2,350 compounds (Green, 1977) including tars and polycyclic hydrocarbons. Thus the presence of styrene is unlikely to play an influential role in the diseases associated with smoking.

5.4 GENERAL CONCLUSION

Styrene is a very important commodity chemical used extensively in the manufacture of numerous polymers and copolymers. It is a component of cigarette smoke and automobile exhaust and may occur at low levels as a natural component of various food types. The highest potential human exposure is in the occupational setting especially in those occupations involving the production of large glass-reinforced polyester products. Lower exposures occur in styrene monomer and polymer production. The general public is exposed to extremely low levels of styrene in ambient air, indoor air, food, drinking water and from cigarette smoke. Exposures resulting from styrene in food packaging material is even lower. For a review of the subject see Miller *et al*, 1994.

6. MEASUREMENT TECHNIQUES AND ANALYTICAL METHODS

6.1 AT THE WORKPLACE

There are a number of methods that can be used to measure the styrene level in the employees breathing zone. Styrene is generally collected in the breathing zone by drawing air (by means of a battery powered pump) through a tube containing a solid sorbent. (The pump unit will need to be certified for use in flammable atmospheres). The solid sorbent can be charcoal or a porous polymer such as Tenax. The styrene is desorbed from the adsorbent by chemical means (charcoal tube) or thermally (charcoal and Tenax tube) and analysed by gas chromatography (GC) using a Flame Ionisation Detector (FID).

Alternatively, a passive sampler can be used. This can be a badge or a tube type. The solid sorbent material in the badge type is normally charcoal while the tube type can contain charcoal or porous polymer (Tenax). Desorption of the badge type sampler is normally by chemical means and the Tenax samples are thermally desorbed. The styrene is analysed by GC.

The styrene content in the sample in mg/m^3 is calculated from the weight of the styrene found in the sample and the volume of air drawn through the tube (pumped sample) or from the uptake rate of styrene for the passive sampler. Uptake rates for specific compounds can be obtained from the suppliers of commercially available passive samplers.

An indication of styrene concentrations in the workplace atmosphere can be obtained by using chemical detection tubes. Air is drawn through the tube using a small hand pump. The contaminant in the air reacts with the chemical in the tube causing a colour change. The length of the colour stain is a function of the air drawn through the tube and the concentration of the contaminant in the air. Such sampling methods are not suitable for monitoring personal exposures to styrene for compliance with exposure standards. In addition many chemical detection tubes are not substance specific.

Some direct reading instruments can be used for measuring styrene in workplace air but will need to be calibrated for styrene. Again such instruments do not normally reflect the 8 h integrated exposure and the results cannot in such circumstances be used to compare against the exposure standard. The result of breathing zone monitoring may not reflect the total exposure to styrene as exposures via skin may occur - although this is a very minor route of exposure. Combined exposures can be monitored by measuring styrene and/or styrene metabolites in body fluids such as urine.

6.2 ENVIRONMENTAL MONITORING

To measure environmental levels of styrene, ambient air is drawn through an appropriate solid adsorbent. The styrene is desorbed by gas purging into a gas chromatograph fitted with FID. Alternately, a more specific identification and quantification can be achieved by a combination of GC-MS technique. Methods for detection have been standardised by a number of institutes - see Toxicological Profile of Styrene, U.S. Department of Health and Human Services (1992).

Using such methods relatively low detection limits (0.01 mg/sample, 0.10 µg/l in water and 4.0 µg/kg in soil) can be achieved.

The most comprehensive survey of environmental exposure to styrene was undertaken by the Office of Acid Deposition, Environmental Monitoring and Quality Assurance, US Environmental Protection Agency. The sampling and analytical procedures developed for the Total Exposure Assessment (TEAM) Study (1987) described in detail by Handy *et al* (1985) are summarised below.

Styrene concentrations in ambient air can be measured by drawing a measured volume of air at approximately 30 ml/min through a glass or stainless steel tube containing Tenax-GCn a porous polymer adsorbent. Sampling periods can be up to 12 h or more providing that the sampling capacity of the adsorbent is not exceeded. The styrene is thermally desorbed and analysed by GC or GC/MS techniques.

6.3 BIOLOGICAL TISSUES

As styrene is distributed throughout the body and deposits in fatty (adipose) tissues the levels of styrene in fat tissue collected from occupationally (Wolf *et al*, 1977, 1978), experimentally (Engstrom *et al*, 1978b) and environmentally (Stanley, 1986) exposed individuals have been measured. In the first of these studies fat tissue samples were collected from workers in a styrene polymerisation plant where exposures were estimated to range from 1 to 5 ppm. Levels of styrene in fat samples collected at various times up to 8 h after the end of exposure varied between 100 to 1,200 ng styrene/g of fat with an average concentration of 430 ng/g. In the study by Engstrom *et al* (1978b) levels of styrene in fat collected from volunteers 21 h after being exposed to 50 ppm styrene for 2 h averaged 2,420 ng styrene/g of fat. In the study described by Stanley (1986) a total of 763 fat tissue samples collected in the US were coalesced into 46 composite tissue samples. These composite samples were then separated by geographical location and stratified into three age groups (i.e. 0-14, 15-44 and >45 years). Styrene was reported to be present in all 46 samples with the highest level (i.e. 122 ng styrene/g of fat) in the youngest age group; the intermediate and oldest groups had levels of 89 and 65 ng/g respectively. The levels reported in the latter study are extremely high considering the low levels of exposure to styrene in ambient air (i.e. approx. 1 ppb) and food (4 µg/d). The biological implausibility of the results reported by Stanley (1986) and the criticism of the study by the National Research Council - for full evaluation see Miller *et al*, (1991) - may have been a factor in the USEPA dropping further studies in the National Human Adipose Tissue Survey; while difficulties in collecting, storing and analysing fatty tissue are obvious limitations to using fat analysis as a routine assay.

Analysis of venous blood using gas chromatography has been examined by a number of authors as a method of monitoring styrene exposures. Astrand *et al* (1974) found that during exposure there was apparently no good correlation between alveolar air and blood concentrations. In a study on 491 styrene polymerisation workers Wolff *et al* (1978) reported that levels of styrene in blood were elevated according to level of exposure although most concentrations were less than 2 ng/ml which was considered to be consistent with an exposure level in the range of 10 ppm. Bartolucci *et al* (1986) found that styreneaemia measured at the end of the workshift appeared to correlate quite well with styrene TWA concentrations. Examination of the figures in this paper suggests that an exposure of about 30 ppm gives a blood concentration of about 180 ng/ml. Blood styrene was also measured by Perbellini *et al* (1993) in 76 workers exposed to styrene and 81 control (non-exposed) individuals. At the end of the workshift (average exposure to styrene approximately 48 ppm) the blood styrene was 1.200 ng/ml; levels in the controls were in the main below 0.5 ng/ml. As described by Guillemin and Berode (1988) when using blood as an estimation of exposure it is necessary to take samples in the first few hours after exposure since levels drop very quickly. Other precautions include ensuring no loss occurs into the headspace above the blood and contact with rubber and plastic must be prevented. Overall data on the precision, accuracy and utility of blood measurements are limited (Guillemin and Berode, 1988).

7. TOXICOLOGY

7.1 TOXICOKINETICS

The occurrence of target organ toxicity is dependent on a host of factors including the distribution of the parent compound and metabolites into and out of the target organ, as well as the rates of formation and degradation of the ultimate toxicant in the target organ. For styrene, this is particularly complex since there are multiple isozymes for the activation of styrene to styrene oxide (e.g., cytochrome p-450) as well as for the inactivation of styrene oxide (e.g., glutathione S-transferase and epoxide hydrolase). There is extensive published information related to the toxicokinetics of styrene. This document incorporates the major conclusions that can be drawn from the available published information, together with preliminary results of unpublished studies currently in press.

7.1.1 Uptake

The uptake of styrene in humans and animals proceeds by all routes of exposure (oral, dermal and inhalation). The most substantial human exposures occur via pulmonary uptake of styrene vapours in occupational settings. Pulmonary absorption has been evaluated in several human studies that show uptake values ranging from 61 to 77% of the inspired air concentration over exposure periods of 2 h (Lof *et al*, 1986a; Wigaeus *et al*, 1983; Wigaeus *et al*, 1984), 6 h (Ramsey and Young, 1978) or 8 h (Fiserova-Bergerova and Teisinger, 1965; Bardodej and Bardodejova, 1970; Wieczorek and Piotrowski, 1985). Respiration rate has a significant effect on uptake, as indicated by a human study in which a 5- to 6-fold increase in pulmonary uptake occurred during conditions of heavy exercise compared to the amount taken up during resting conditions (Engstrom *et al*, 1978a). However, the percent of styrene absorbed from the respiratory system appears to be quite constant over a broad range of exposure concentrations since, at air concentrations of 20, 40, 100 or 200 mg/m^3, the average retention over an 8-h period in human volunteers was found to be 71.2%, 71.6%, 72.1% and 69.5%, respectively (Wieczorek and Piotrowski, 1985). Assuming 70 % absorption and an inspired air volume of 10 m^3 during an 8-h work day, the estimated total styrene absorption would be 1491 mg during an 8-h time-weighted average occupational exposure to 50 ppm (the current OSHA Permissible Exposure Level for styrene).

The rate of absorption of liquid styrene through the skin is apparently quite low, in the order of 0.06 mg/cm^2/h based on results of a human study by Berode *et al* (1985). This study showed conclusively that older studies by Dutkiewicz and Holina (1969) seriously overestimated the uptake of styrene through the skin. Dermal absorption of styrene vapours is negligible, as shown in studies by Riihimaki and Pfaffli (1978) and Wieczorek (1985), in which the authors concluded that skin absorption of vapours was only about 2% of that observed from the respiratory system.

There have apparently been no human studies in which absorption of styrene from the gastrointestinal tract has been systematically evaluated. However, studies in rats indicate that absorption from the gastrointestinal tract is rapid and virtually complete. Studies by Withey (1976), for example, showed that peak blood levels of styrene occurred within

minutes following an oral dose in aqueous solution, and in less than 2 h after an oral dose in oil. Approximately 90-95% of an oral dose of radiolabelled styrene was recovered as urinary metabolites, indicating nearly complete absorption (Sauerhoff and Braun, 1976; Plotnick and Weigel, 1979).

7.1.2 Distribution

Human styrene tissue distribution studies are limited to quantitative analyses of styrene in blood or adipose tissue samples. Wolff *et al* (1977) detected styrene in adipose tissue biopsies from 13 of 25 workers exposed to styrene within the previous 3 days, but not in samples from workers removed from exposure for more than 3 days. Engstrom *et al* (1978b) measured styrene concentrations in adipose tissue biopsies from several human volunteers after a 2-h experimental inhalation exposure to 50 ppm styrene vapours. The concentration of styrene in adipose tissue averaged 2420 ng/g during the first 21 h after exposure, while expired air concentrations averaged approximately 1 mg/m^3 when measured 2 to 4 h after exposure. In this study, the styrene concentration in adipose tissue decreased exponentially after exposure, with an estimated half-life in adipose tissue of 2-4 days. Styrene blood concentrations ranging from approximately 1 to 90 ppb were detected by Wolff *et al* (1978) in samples from styrene polymerisation workers exposed to styrene air concentrations estimated to be in the range of 10 ppm (based on urinary mandelic acid measurements). Additional studies of styrene polymerisation workers by Wolff *et al* (1977) indicated that styrene concentrations in fat varied from 100 to 1200 ng/g when measured at various time points up to 8 h after styrene exposures estimated to range from 1 to >5 ppm. Ramsey and Young (1978) found that styrene blood levels rose rapidly and then levelled off at about 0.9 µg/ml in male human volunteers exposed to 80 ppm styrene vapours for 6 h. The authors noted that at exposure concentrations below 200 ppm, styrene is very efficiently cleared from the body, and will not continue to accumulate upon repeated exposure. Studies with human volunteers by Wigaeus *et al* (1983; 1984) indicated that the amount partitioned into adipose tissue represented 8% of the styrene taken up from inspired air.

Studies with laboratory animals indicate that intravenously administered styrene is rapidly distributed to major organs, with relatively higher concentrations in the brain than in heart, liver, lung, spleen and kidney (Withey and Collins, 1977). Intraperitoneal administration of ^{14}C-styrene resulted in higher concentrations of radiolable in fat than in other tissues (Lof *et al*, 1983). Inhalation exposures of rats to air concentrations ranging from 50 to 2,000 ppm for 5 h resulted in at least a 10-fold higher concentration of styrene in the perirenal fat than in any other tissue (Withey and Collins, 1979). After oral exposure, peak tissue concentrations were reached within 2-4 h (Plotnick and Weigel, 1979). Carlsson (1981) exposed male Sprague-Dawley rats to approximately 44 or 240 ppm radiolabeled styrene for one to eight h. Liver, kidneys and fat were found to have high concentrations of the radiolabel, and the author estimated the half-life for styrene in fat to be about 2 h.

Clear changes in the adipose tissue concentration of styrene have been noted in rats exposed to 300 ppm styrene during an 11-wk period, with perirenal fat concentration at 11 wk being less than half of the peak value (1567 nmol/g) measured after 4 wk of exposure (Savolainen and Pfaffli, 1977). Elovaara *et al* (1979) also reported higher perirenal fat concentrations of

styrene in rats after 4 wk of exposure to 300 ppm as compared with longer periods of treatment; a roughly inverse relationship between fat styrene concentration and overall hepatic monooxygenation activity was noted. The lower concentration of styrene in fat over the course of time in these studies is considered to reflect changes in biotransformation during the exposure period. Teramoto and Horiguchi (1981) reported that after a single inhalation exposure to styrene for 4 h (500 to 1,000 ppm) the apparent distribution of styrene in male JCL rats decreased in the order: adipose tissue>>liver>brain>kidney>blood=spleen>muscle. The biological half-life of styrene was about 6 h for adipose tissue, and 2 h for the other tissues. Almost the same results were obtained in rats following a single intraperitoneal injection of 350 mg/kg. Repeated 4-h inhalation exposures (700 ppm for 5 d) showed that the blood and adipose tissue concentrations of styrene were similar to those after a single exposure, with no indication of accumulation. The authors concluded that bioaccumulation of styrene does not occur in workers repeatedly exposed to styrene when exposures are maintained below 100 ppm.

7.1.3 Biotransformation

The metabolic fate of styrene is shown in Figure 1. In both humans and rodents, styrene is initially biotransformed to styrene-7,8-oxide (SO) via microsomal cytochrome P-450 monooxygenases. Once formed, SO is either conjugated with glutathione via cytosolic enzymes or converted via microsomal epoxide hydratase to styrene glycol. Styrene glycol is in turn further metabolised into the urinary metabolites mandelic acid (MA), phenylglyoxylic acid (PGA), and benzoic acid or its glycine conjugate hippuric acid.

MA and PGA are the two major urinary metabolites of styrene in humans (Wolff *et al*, 1978; Guillemin and Bauer, 1979; Korn *et al*, 1987; Lof *et al*, 1986b; Bardodej and Bardodejova, 1970; Wigaeus *et al*, 1983), while in rodents hippuric acid is one of the main urinary metabolites of styrene (Ohtsuji and Ikeda, 1971). For humans, MA and PGA have been reported to represent 33.6% of the total styrene uptake at the end of a 14 h exposure period, 58% within 28 h of the start of exposure, and 86% during four days following exposure.

A minor detoxification pathway for styrene-7,8-oxide in humans involves conjugation with glutathione (GSH) via cytosolic glutathione-S-transferases, followed by further biotransformation of the conjugate into mercapturic acids which are excreted in the urine (Malonova and Bardodej, 1983). The GSH pathway may be much more important in rodents than in humans as a detoxification route for SO, especially at high exposure concentrations. For example, Seutter-Berlage *et al* (1978) identified three sulphur-containing metabolites (glutathione derivatives) in the urine of rats given an intraperitoneal injection of styrene (250 mg/kg in sesame oil); these metabolites amounted to approximately 10% of the administered dose of styrene. These results were consistent with another study with rats and rabbits that also showed substantial amounts of mercapturic acid derivatives in the urine after dosing with styrene or styrene oxide (James and White, 1967).

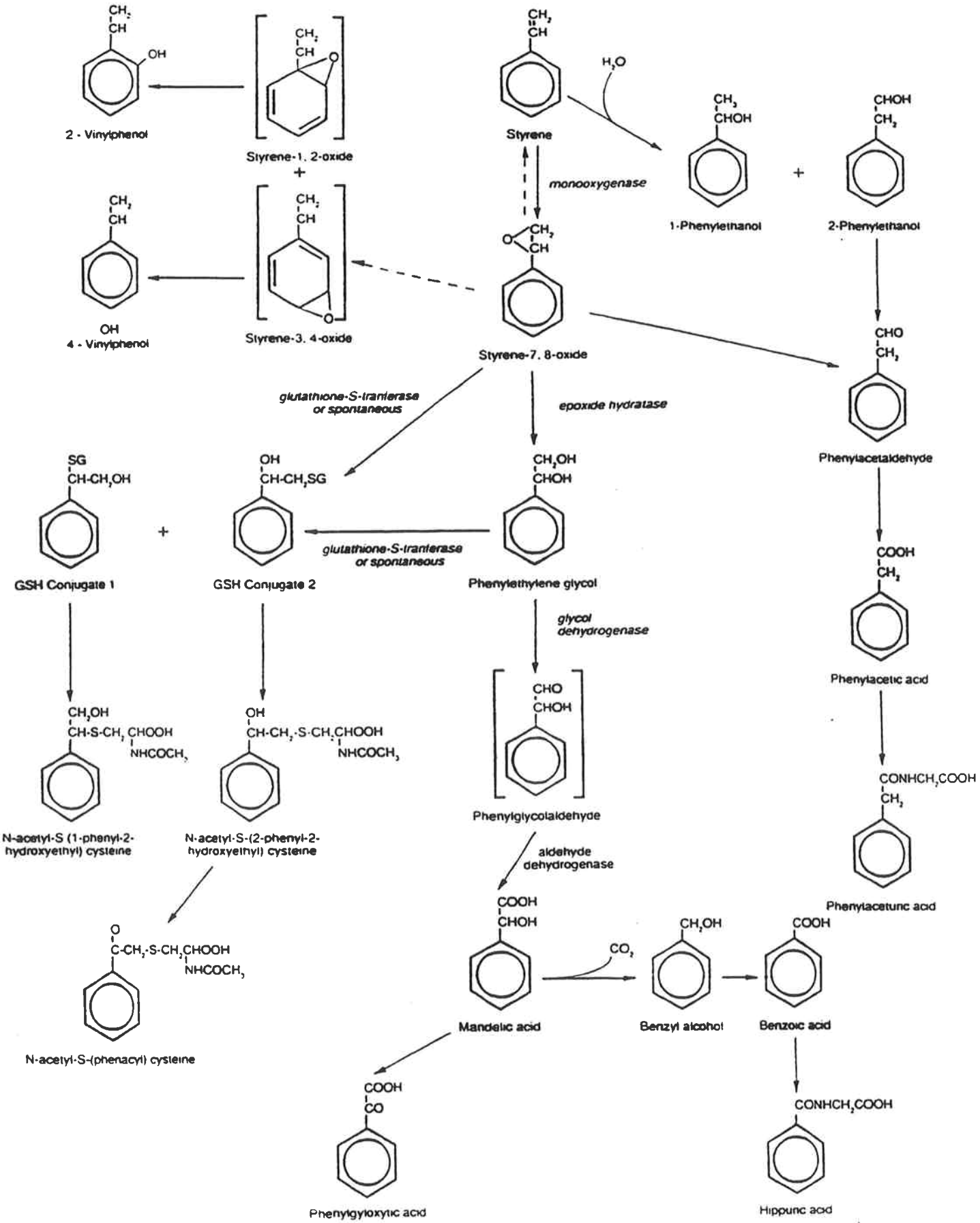
An additional minor metabolic pathway involves the production of vinylphenols, possibly via arene oxide intermediates. Bakke and Scheline (1970) reported that, in rats, 0.1% of an oral dose of styrene (100 mg/kg in propylene glycol) was eliminated in urine as 4-vinylphenol.

Conjugates of both 2-vinylphenol and 4-vinylphenol have been reported at low levels in the urine of rats injected intraperitoneally (dose not specified) with styrene (Hiratsuka *et al*, 1982). Pantarotto *et al* (1978) also reported that 4-vinylphenol was a minor metabolite in rats given an intraperitoneal injection of styrene, although again the dose of styrene was not specified. *in vitro* covalent binding studies with proteins of the rat liver endoplasmic reticulum also suggested that an arene oxide may by formed as a minor metabolite of styrene (Pantarotto and Blonda, 1984). In humans, 4-vinylphenol has been detected in the urine of workers exposed to a mean styrene concentration of 130 ppm; the 4-vinylphenol metabolite amounted to only about 0.3% of the amount of urinary mandelic acid, indicating that the ring oxidation is a minor metabolic pathway (Pfaffli *et al*, 1979; Pfaffli *et al*, 1981). However, the possibility that 4-vinylphenol in urine may have been due to an impurity in the styrene has not been excluded.

In rats, 1-phenylethanol and 2-phenylethanol have been reported as minor styrene metabolites in urine (Bakke and Sheline, 1970), but these metabolites have apparently not been reported in humans. Delbressine *et al* (1981) reported that phenylaceturic acid (apparently produced via conjugation of glycine with phenylacetic acid) was also a minor styrene metabolite in rats. These authors suggested that phenylacetic acid was produced by further oxidation of phenylacetaldehyde, which in turn resulted from oxidation of 1- and 2-phenylethanol or intramolecular rearrangement of styrene oxide.

Belvedere *et al* (1977) evaluated the kinetic behaviour of microsomal styrene monooxygenase and SO hydratase in male Sprague-Dawley rats, Swiss mice, New Zealand rabbits, and Dunkin-Hartley guinea pigs. The ratios of the apparent K_m values of SO hydratase to styrene monooxygenase (K_m hydratase/K_m monooxygenase) were found to be 18.2, 6.4, 4.0 and 4.0 for the mouse, rabbit, rat and guinea pig, respectively. The mouse, in comparison to other species, is far more efficient in forming the epoxide than in detoxifying it. The mouse would therefore be expected to be far more sensitive than the other species, assuming that styrene toxicity was related primarily to the metabolite SO. The authors suggested that the affinity of SO for the hydratase may be the rate limiting step for the overall metabolic transformation of styrene, and that it is the speed of hydration of SO and not its formation that determine the rate at which it is detoxified. Subsequent studies by the same group showed that both styrene monooxygenase and SO hydratase activities were present in liver, heart, lungs, spleen and kidneys of both male and female Sprague-Dawley rats, CD1 mice, New Zealand rabbits, and Dunkin-Hartley guinea pigs (Cantoni *et al*, 1978). The capacity of the liver to form and detoxify SO was higher than for the other tissues in both sexes of all species considered. There were no pronounced sex differences in hepatic enzymatic activities from the four species considered. Consistent with the K_m values previously reported, the ratios of SO hydratase to styrene monooxygenase activities were far lower in mouse tissues than for rats, with the difference being especially pronounced in lung tissue. The hydratase/monooxygenase ratio was also especially low in rabbit lungs. Based on this observation, the authors speculated that the mouse and rabbit lung might be especially sensitive to the toxic action of SO.

Figure 1: The Metabolic Fate of Styrene in Human and Rodents



Tissue and species differences in the enzymes involved in SO metabolism have also been shown by Pacifici *et al* (1981). Using [7-3H] SO as substrate, GSH S-transferase and SO hydratase activities were identified in the liver, lung and kidney of nine species, including humans. In all species, the activities of both enzymes were higher in the liver than in the lung or kidney. The baboon had the highest hepatic SO hydratase activity (31 nmol/mg/min) while the mouse had the lowest hepatic activity of this enzyme (1.9 nmol/mg/min); the human SO hydratase activity (12.7 nmol/mg/min) was intermediate between the mouse and baboon. Rodent species had higher GSH S-transferase activities than non-rodent species, with mouse liver having much higher activity (149 nmol/mg/min) than in rats (87 nmol/mg/min) or in humans (25 nmol/mg/min). The authors concluded that in rodents SO should be preferentially detoxified by conjugation with glutathione in all tissues, whereas in non-rodent species both conjugation and hydration should play a significant role.

Mendrala *et al* (1993) also evaluated the *in vitro* activities of enzymes involved in the formation and degradation of SO in liver and lung tissues from rats and mice, and human hepatic tissue. Based on the V_{max} for styrene epoxidase activity and the relative liver and body size, mice were found to have the greatest capacity and humans the lowest capacity to form SO from styrene. Human epoxide hydratase was found to have a greater affinity (i.e., lower K_m) for SO than epoxide hydratase from rats and mice, indicating that human liver is more effective than rodent liver in hydrolysing low levels of SO formed from styrene. Assuming that repeated-dose styrene toxicity is due primarily to SO, these *in vitro* studies confirm the above suggestions that mice are more sensitive to styrene than rats, and suggest that the rodents would be more sensitive than humans.

Ryan *et al* (1976) studied the *in vitro* metabolism of SO by hepatic and extrahepatic subcellular fractions from various rodent species (rats, rabbits and guinea pigs) and in isolated perfused rabbit lung and rat liver preparations, using 8-^{14}C-SO as substrate. In all three species the highest enzymatic activities of glutathione (GSH) S-transferase and epoxide hydratase were found in the liver. The activities of both enzymes were also quite high in the kidneys of all three species, while lung, skin, and intestinal mucosa activities were comparatively low. Rat testis also had substantial activities of both GSH transferase and epoxide hydratase (testes of rabbits and guinea pigs were not evaluated). Rats and guinea pigs had higher GSH S-transferase activity in both liver and kidney than the rabbit.

In the isolated, perfused rat liver and rabbit lung preparations, conjugation with glutathione was a major metabolic pathway; however, significant amounts of diol were also formed in each instance. In the rat liver, 27-40% of the administered SO was excreted via the bile as a glutathione derivative [S-(l-phenyl-2-hydroxyethyl) glutathione]. No significant covalent binding of radiolabelled SO was detected in the livers used in these organ perfusion experiments.

The detoxification of SO by human liver GSH S-transferase has been studied by Pacifici *et al* (1987). GSH S-transferase activity in adult human liver is known to reside in several basic isozymes with isoelectric points between pH 8 and 10, and one near neutral form (GSTμ) with an isoelectric point between pH 6 and 7. The GSTμ form is present in about one half of the population, and is known to be more active than the basic forms in the conjugation of glutathione with epoxides such as SO (Warholm *et al*, 1981). Individuals lacking the GSTμ

isozyme may have a lower capacity to detoxify SO than those who have such an isozyme, although in general the GSH S-transferase pathway is believed to be of minor importance in humans in comparison to the epoxide hydratase pathway.

Stereochemical considerations are involved in the metabolism of styrene (Watabe *et al*, 1981; 1982a,b; Delbressine *et al*, 1981; Korn *et al*, 1987; Foureman *et al*, 1989). The initial metabolic step in the metabolism of styrene is the microsomal oxidation of the olefin double bond by cytochrome P-450, resulting in the formation of both R- and S-7,8-styrene oxide. Both epoxide hydrolase and glutathione transferase enzymes possess stereoselective preference for the SO enantiomers. Studies by Drummond *et al* (1989) demonstrated that, in both rats and human volunteers, MA formed from styrene was racemic whereas only the R-enantiomer of MA was excreted after ethylbenzene exposure. The stereochemical considerations may be important in the toxicity of styrene since, for example, there are data that show that R-styrene oxide is a stronger mutagen than the S-form in the Ames test. Those data indicate that enantioselective preference could lead to a difference in susceptibility to styrene (Pagano *et al*, 1982; Karels *et al*, 1991).

Induction of the mixed function oxidase (MFO) system by treatment of rats with phenobarbital has been shown to increase the rate of metabolism of styrene. By contrast, the rate of styrene metabolism is decreased by administration of SKF-525A, an inhibitor of the MFO system, or by co-administration of other solvents requiring metabolism (Ikeda *et al*, 1972; Ohtsuji and Ikeda, 1971; Vainio and Zitting, 1978; Ikeda and Hirayama, 1978). Comparison of the binding parameters for the interaction of styrene with non-induced, phenobarbital-induced, and 3-methylcholanthrene-induced microsomes indicated that styrene is predominantly bound by cytochrome P-450 and not by cytochrome P-448 (Vainio and Zitting, 1978).

A significant amount of work has been conducted investigating the ability of styrene or styrene oxide to induce their own metabolism. Parkki *et al* (1976) evaluated the effects of styrene, SO and styrene glycol, on xenobiotic metabolising enzymes in rat liver. Intraperitoneal (ip) doses of 500 mg/kg styrene in corn oil daily for three or six days was reported to double the activities of microsomal p-nitroanisole-O-demethylase and epoxide hydratase (SO as substrate), whereas the activity of aryl hydrocarbon hydroxylase was practically unaffected. Non-statistically significant increases in cytochrome c reductase activity and cytochrome P-450 levels were noted only after six doses of 500 mg/kg. Glucuronyl transferase activity was not affected by styrene if measured from native microsomes. However, when microsomes were pretreated with either digitoxin or trypsin, an approximate doubling of the glucuronyl transferase activity was noted for rats given six doses of 500 mg/kg styrene. The glycine conjugation route was not affected by ip administration of 1,000 mg/kg styrene for 3 days. A single dose of 375 mg/kg SO resulted in a significant decrease in the activities of benz-[a]-pyrene hydroxylase and p-nitroanisole-O-demethylase and in cytochrome P-450 content. Conversely, epoxide hydratase and NADPH cytochrome c reductase activities were not significantly altered by SO. Styrene glycol did not significantly alter the activities of the various xenobiotic metabolising enzymes. The authors noted that styrene had differential effects on epoxide hydratase and cytochrome P-450, although epoxide hydratase is known to be located in the microsomal membrane intimately linked with cytochrome P-450 (Oesch *et al*, 1972).

They suggested that the induction of cytochrome P-450 is not under the same control as that of epoxide hydratase. The enhancement of the activity of epoxide hydratase after styrene administration was thought to suggest that the rodent liver may be able to increase its capacity to metabolise SO in response to chronic styrene exposure.

In contrast to the styrene-induced enhancement of epoxide hydratase activity, additional studies by Marniemi and Parkki (1975) indicated that the glutathione conjugation pathway was unaffected by styrene. Three daily ip styrene administrations of 1,000 mg/kg resulted in no enhancement of glutathione transferase activity in male Wistar rats, while pretreatment with phenobarbital resulted in a significant increase (about 56%) in SO conjugation with glutathione.

Studies by Lambotte-Vandepaer *et al* (1979) indicated that the catalytic properties of several liver microsomal enzymes were modified by ip administration of styrene to rats, with the effect being almost exclusively limited to the K_m of those enzymes. A single ip dose of 500 mg/kg of styrene decreased the K_m of benzo(a)pyrene hydroxylase, aldrin expoxidase, and epoxide hydratase (SO as substrate), while having no apparent effect on the K_m of styrene epoxidase (styrene as substrate); V_{max} of these enzymes was not altered by the single ip styrene dose. The authors noted that styrene could possibly change the equilibrium between the various metabolic pathways by modifying the affinity of the substrates of those enzymes.

Sandell *et al* (1978) studied the effects of inhalation and cutaneous exposure to styrene on the xenobiotic metabolising enzymes of the rat. Male Wistar rats were exposed to 450 ppm styrene by inhalation (8 h/d, for seven consecutive d), or by cutaneous administration (500 or 3,000 mg/kg/d for seven consecutive days). Styrene inhalation increased the activities of hepatic epoxide hydratase and glucuronyl transferase, as well as ethoxycoumarin O-deethylase in the kidney. Cytochrome P-450 content in the liver and the activities of NADPH cytochrome c reductase, benzpyrene hydroxylase and GSH S-transferase in the liver and kidney were not altered. No treatment related changes of enzyme activities were found in the lung, and there was no indication of enzyme induction after cutaneous administration of styrene. In fact, liver epoxide hydratase activity was found to be depressed after cutaneous administration of styrene.

The effects of styrene inhalation exposure on the xenobiotic metabolising enzymes of male Wistar rat liver and kidney were also evaluated by Vainio *et al* (1979). Intermittent inhalation exposure of adult male rats to 300 ppm styrene vapours, 6 h/d, 5 d/wk for up to 11 wk enhanced the activities of ethoxycoumarin O-deethylase, epoxide hydratase, and glucuronyl transferase in the liver and kidneys. Cytochrome P-450 levels of hepatic microsomes were doubled after 2-wk exposure and remained significantly higher than controls after 11 wk. In kidneys, cytochrome P-450 levels were significantly elevated only after 11 wk of exposure. Increased ethoxycoumarin O-deethylase activity was noted in both liver and kidney after only 2-wk of exposure, whereas significant enhancement of glucuronyl transferase activity was not observed until after the 6th wk of exposure. Epoxide hydratase activity was not determined after 2 wk, but was found to be higher than for controls when measured after 4-, 8-, and 11-wk exposures (statistically significant only at 11 wk).

In contrast to the enhancements in hepatic enzymatic activities, further studies by the same group indicated that a single exposure to 500 ppm styrene for 24 h resulted in substantial decreases in pulmonary cytochrome P-450 and in the activities of various pulmonary monoxygenase enzymes (Elovaara *et al*, 1990). These pulmonary enzymatic changes were associated with decreased pulmonary NPSH levels, as well as a 70-fold increase in urinary thioether excretion. The significance of these observations following a 24 h exposure regimen is uncertain, since only slight decreases (mostly not statistically significant) were noted following a more relevant exposure regimen (500 ppm, 5 h/d, for 5 d). The authors concluded that the biochemical changes were unlikely to be caused by direct irritation, but substantiation of that conclusion was not provided.

Das *et al* (1981) reported a dose-dependent increase in hepatic aminopyrene- and ethylmorphine-N-demethylase activities as well as arylhydrocarbon hydroxylase and aniline hydroxylase activities in rats given high oral doses of styrene (450 or 900 mg/kg for 7 d). These investigators also reported that GSH S-transferase activities were lower in rats given 450 or 900 mg/kg styrene than in control animals.

Mendrala *et al* (1993) evaluated the *in vitro* activities of enzymes involved in the formation and degradation of SO in liver and lung tissues from rats and mice with and without prior exposure to styrene. Prior exposure of rats (1,000 ppm, 6 h/d for 4 d) or mice (600 ppm, 6 h/d for d) to styrene had no apparent effect on styrene epoxidase activity or GSH S-transferase activity in either liver or lungs of rats or mice. However, there was a 1.6-fold increase in the activity of hepatic SO hydratase in rats pre-exposed to styrene; this was consistent with the observation previously reported by Parkki *et al* (1976). Prior exposure to styrene had no apparent effect on K_m or V_{max} of hepatic styrene epoxidase in either rats or mice.

Co-administration of ethanol has been reported to modify the styrene-induced biotransformation changes in rat liver and kidney (Elovaara *et al*, 1979). Male Wistar rats were exposed either to 300 ppm styrene (6 h/d, 5 d/wk) or to a 15% solution of ethanol in drinking water, either alone or in combination, for up to 17 wk. The activities of 7-ethoxycoumarin O-deethylase and 2,5-diphenyloxazole hydroxylase in both liver and kidneys were increased more by ethanol ingestion than by styrene inhalation. The activities of these two enzymes appeared to be enhanced by an additive effect when ethanol and styrene were given in combination. Hepatic SO hydratase activity was found to be virtually unaffected by the styrene exposures, and hepatic NADPH-cytochrome c reductase activity was reduced both in styrene and in ethanol-treated rats. Hepatic glucuronyl transferase activity was enhanced slightly in styrene-treated rats, as well as in rats given styrene and ethanol in combination. Small increases in liver cytochrome P-450 were noted, which proved to statistically significant only in the styrene-ethanol group. However, the binding affinity of styrene for hepatic cytochrome P-450 was reported to be increased after styrene inhalation.

7.1.4 Elimination

Human studies have shown that 90-97% of absorbed styrene is eliminated as urinary metabolites (Guillemin and Bauer, 1978; Ramsey and Young, 1978), while only a small

fraction is eliminated as unchanged styrene in urine or expired air (Stewart *et al*, 1968; Imbriani *et al*, 1985). The post-exposure urinary elimination of MA is apparently biphasic, with the half-time for the first phase (<20 h after exposure) being about 4-9 h, and the second phase (>20 h after exposure) about 17-26 h (Guillemin and Bauer, 1979; Engstrom *et al*, 1976). The urinary elimination of PGA seems also to be biphasic, with the half-time for the first phase (<50 h after exposure) being about 10 h, and the second phase (50-200 h after exposure) about 26 h (Guillemin and Bauer, 1979; Caperos *et al*, 1979). Studies with human volunteers indicate that the half-life of styrene in adipose tissue is about 2-4 d (Engstrom *et al*, 1978).

Excretion have been shown to be substantially altered in human volunteers exposed to both styrene and ethanol (Wilson *et al*, 1983). One hour after administration of ethanol, blood MA levels were 56% of the levels found during the alcohol-free control styrene exposure, and this was associated with a 15-fold elevation in blood levels of phenylethane 1,2 diol (styrene glycol), the metabolic precursor of MA. The authors suggested than the changes in MA kinetics were the result of inhibition of the oxidation of this diol subsequent to changes in NAD+/NADH ratio produced by ethanol metabolism. Berode *et al* (1986) confirmed the influence of ethanol on MA kinetics, and showed that PGA kinetics is less influenced than MA.

Animal studies have shown that the elimination of styrene from tissues is quite rapid, with only very low levels (< 1µg/g) remaining 24 h after oral dosing (Plotnick and Weigel, 1979). Styrene is removed from the blood via partitioning into adipose tissue as well as by metabolism; the elimination from fat lags behind other tissues, but repeated exposures have been shown not to result in accumulation (Pantoroto *et al*, 1980; Teramoto and Horiguchi, 1979; 1981). After cessation of exposure, release of styrene from adipose tissue ensues as blood styrene concentrations decline due to metabolism and clearance processes. There is evidence that the metabolism of styrene in animals is saturable at high doses or exposure concentrations (Ramsey and Young, 1978; Young *et al*, 1979; Teramoto and Horiguchi; 1979; 1981). At these high doses where the metabolic capacity of the liver and other tissues is overwhelmed, the blood styrene concentration begins to rise disproportionately, deposition into adipose tissue increases, and styrene elimination in expired air increases.

7.1.5 Pharmacokinetics

The pharmacokinetics of styrene in rats and humans were described by Ramsey and Young (1978). Human volunteers were exposed to 80 ppm styrene for 6 h, and rats were exposed to concentrations ranging from 80 to 1200 ppm for 6 h. For both rats and humans exposed to 80 ppm, the clearance of styrene from blood could be described with a two-compartment linear pharmacokinetic model. However, when rats were exposed to higher concentrations between 200 and 600 ppm, the clearance process from blood became saturated. For example, the maximum blood concentration of styrene in rats increased almost 80-fold when the exposure concentration was increased 15-fold from 80 ppm to 1200 ppm. The results suggest that the rat may be a good animal model for understanding the pharmacokinetics and toxicity of styrene, at least at concentrations up to 80 ppm. Andersen and Ramsey (1983) developed a physiologically-based pharmacokinetic (PB-PK) model that accurately described the non-linear behaviour of styrene in rats exposed to high concentrations. This model was

also able to accurately represent the data from humans exposed to 80 ppm styrene. Expanding on this work, Ramsey and Andersen (1984) simulated routes other than inhalation, and applied the model to interspecies comparisons. The model predictions indicated that styrene metabolism is saturated at styrene exposure concentrations greater than 200 ppm in rats, mice and humans. Additional studies by Andersen *et al* (1984) focused on the pharmacokinetics in rats under conditions in which the metabolism of styrene was either induced or inhibited. Fischer 344 rats were exposed to styrene concentrations ranging from 100 to 2,000 ppm. Subgroups of rats were pre-treated with pyrazole (an inhibitor of styrene metabolism), phenobarbital (an inducer), or styrene (1,000 ppm, 6 h/d, 4 d). Pre treatment with pyrazole essentially abolished styrene metabolism, while phenobarbital increased V_{max} about 6-fold, and styrene increased V_{max} by a factor of 2.

Extensive information has been developed regarding the kinetics of styrene-7,8-oxide (SO) as an intermediate metabolite of styrene. The levels of styrene-7,8-oxide (SO) in blood are expected to be very low as a result of the rapid rate at which it is hydrolyzed and conjugated. Lof *et al* (1984) reported measurements of SO in several tissues of mice intraperitoneally dosed with radiolabelled styrene. SO was also reported at very low levels ranging from 0.02µg/ml to 0.05 µg/ml in venous blood samples from several human subjects exposed to styrene (Wigaeus *et al*, 1983; Lof *et al*, 1986a, 1986b). However, the *in vivo* measurements of SO in these studies involved analytical methods in which SO was detected only indirectly via acid hydrolysis to styrene glycol.

Due to the potential for analytical errors with the indirect methods, extremely sensitive direct analytical methods have recently been developed (Langvardt *et al*, 1991; Kessler *et al*, 1990). These direct methods were in close agreement in showing that the *in vitro* half-life of SO in rat whole blood was in the range of 24-26 minutes at 37°C, at an initial blood concentration of approximately 10 µg/g. The unexpectedly long half-life of SO in whole blood *in vitro* indicates that SO is not as reactive as was hitherto assumed. Kessler *et al* (1990) found that at steady state, the SO blood concentrations ranged from 0.008 µg/ml to approximately 0.45 µg/ml in male Sprague-Dawley rats exposed to styrene air concentrations of 20 ppm to 800 ppm, respectively. Near maximum SO steady state blood levels were attained in rats exposed to 260 ppm styrene, and exposure to higher styrene concentrations resulted in very little increase in SO blood levels. Additional studies by the same group showed that SO blood levels in B6C3F1 mice were approximately the same as in rats at lower styrene exposure concentrations ranging between 20 and 260 ppm. At styrene exposure concentrations above 260 ppm, however, the SO blood concentrations in male mice increased dramatically to approximately 6 µg/ml in animals exposed to 800 ppm (Filser *et al*, 1991). The observed differences in SO blood levels in rats and mice exposed to styrene in these studies are therefore consistent with the differences in sensitivity of the two species previously discussed.

Nolan *et al*(1991), using the methodology developed by Langvardt *et al* (1991), found that a single SO oral dose of 550 mg/kg resulted in peak blood levels of 12.9 µg/g in rats. During the first 10 h after rats were given a single 500 mg/kg oral dose of styrene, the SO blood levels were relatively constant between 0.093 and 0.172 µg/g, confirming that the metabolism of styrene was saturated. Thus, the blood SO concentrations in rats in this study represent the highest concentrations of SO that can occur in rats exposed orally to styrene. While the

peak SO blood concentrations were much lower (<2%) after oral administration of 00 mg/kg of styrene than after 550 mg/kg of SO: the area under the blood SO concentration-time curve (AUC) for styrene was almost half of the AUC for SO.

Csanady *et al* (1994) developed a PB-PK model incorporating consideration of SO being an intermediate metabolite of styrene. Consistent with the previously discussed experimental animal data, the PB-PK model shows pronounced species differences in internal SO dosimetry following exposure to styrene. For example experimental data show, and the model predicts, that SO blood levels in the mouse and rat are very similar when exposed to styrene at concentrations ranging between 20 and 260 ppm. Above this exposure level the SO blood concentrations in the mouse, as compared to the rat, increases dramatically. For example blood SO levels measured in the mouse exposed at a styrene concentration of 800 ppm is at least an order of magnitude higher than that measured in the rat (ECETOC Special Report No. 2, 1992). Interspecies differences in metabolism (i.e. the mouse detoxification mechanism being overwhelmed at higher exposure levels) are considered to be responsible for this phenomenon. Thus with high exposures to styrene, the detoxification system in rodents becomes overwhelmed (mouse more so than the rat) resulting in reduced elimination and a compensatory increase in internal levels of SO. Because of differences in metabolism between rodents and man (i.e. studies by Mendrala *et al* (1993) show that mice have the greatest capacity to form SO from styrene whereas humans have the lowest; conversely humans have the greatest capacity to detoxify SO while rodent liver is the least effective at hydrolysing the oxide) rodents have far higher internal SO doses than humans at any given external styrene exposure.

In summary, styrene is well absorbed by all routes of exposure. After absorption, styrene is partitioned to the body fat, but bioacculmulation does not occur in humans at styrene exposure concentrations lower than 100 ppm. Styrene is rapidly and extensively metabolised, and in humans mandelic acid and phenylglyoxylic acid are the two major urinary metabolites. The metabolism of styrene proceeds via styrene-7, 8-oxide (SO), but recent studies have shown that there are pronounced species differences in the internal SO dosimetry following exposure to styrene. Rodents have a greater capacity than humans to form SO from styrene, while humans are more effective than rodents in detoxifying low levels of SO.

7.1.6 Biological Monitoring

***7.1.6.1 Urinary Metabolites*:** A number of studies have been undertaken to evaluate the use of measuring the urinary metabolites of styrene mandelic acid (MA) and/or phenylglyoxylic acids (PGA) as a biomonitoring method for assessing occupational exposure to styrene. Analysis of MA and PGA in urine poses no real analytical problems with liquid chromatography, gas chromatography and isotachophoresis all having been shown to be suitable methods of analysis (Guillemin and Berode, 1988). There are however some considerations when using measurements of urinary MA and PGA as estimates of styrene exposure two of the most important being when to collect the urine and storage of the sample.

Maximum excretion of MA occurs at the end of the shift although alcohol consumption, which inhibits the transformation of styrene glycol into MA, can delay excretion for several h. Based

on published studies Guilleman and Berode (1988) showed that the mandelic acid concentration in end-shift urine exposed 8 h to 100 ppm styrene varied between 2,300mg/g creatinine to 760 mg/g creatinine; although the majority of the studies reported levels of 1,500 and above. The correlation between the exposure concentration in ppm (Cexp) and the concentration of MA in end-of-shift urine (MA expressed as mg MA/g creatinine) is given by the equation:

Cexp = 65MA-3.6

All the figures are corrected for creatinine since this improves significantly the correlation with exposure (Engstrom *et al* 1976).

Storage of the samples is critical. Urine should be kept refrigerated or frozen since PGA is prone to decomposition at room temperature. Kivisto *et al* (1993) suggested that for accurate analysis, using either diode array detection technique or gas chromatography, it is essential to store the urine samples frozen together with an external quality control sample.

As PGA is an oxidative metabolite of MA measurement of this acid is considered complementary to MA estimations especially since the ratio of MA/PGA may be dependent on exposure intensity (Guillemin and Berode, 1988). As PGA excretion represents 33% of the styrene absorbed so MA and PGA corresponds to about 90% of styrene retained in the body. Several authors have advocated that the measurement of MA and PGA in urine sampled at the end of the shift or the next morning provides the most reliable biological indication of styrene exposure (Droz and Guillemin, 1983, Bartolucci *et al*, 1986 and Guillemin and Berode, 1988).

Despite the number of studies on urinary excretion of styrene metabolites there is still no common agreed monitoring procedure with, as described above, some authors recommending measurement of MA or calculating the sum of MA and PGA at the end of the shift, others propose PGA measurements while still others see measurements of PGA and MA 16 h after exposure as the optimum method. Another problem is the large interindividual variability in MA and PGA excretion caused by a variety of factors including interference of other solvents while drug and alcohol consumption can also affect excretion of the metabolites. Gobba *et al* (1993) has proposed that measuring styrene in urine may help overcome some of these problems since a study on 214 individuals indicated that measurement of urinary styrene represents a good exposure index for exposure. Further studies are obviously required to examine the reproducibility and robustness of this method.

The determination of minor metabolites such as styrene mercapturic acids have also been measured in exposed individuals (Norstrom *et al*, 1992) but such investigations are provisional and can not be considered, at present, to represent a biomonitoring tool. At present it would appear that the most documented biological test for styrene exposure is measuring MA in urine at the end of the wok shift or the sum of MA and PGA measured the following morning. Some of the difficulties and potential problems of using the urinary metabolites as a biological indicator for styrene exposure have been described briefly above and are addressed more fully by Guillemin and Berode (1988). Because of such problems it is possible that some

styrene exposure estimates based on urinary metabolites metabolism may not reflect accurately occupational exposure.

7.1.6.2 Macromolecular Binding Studies: Macromolecular binding studies have become increasingly important as potential biomonitoring tools to estimate the degree of exposure to reactive substances. Protein adducts in easily accessible body fluids (e.g. haemoglobin adducts) may be a useful biomonitoring tool to assess cumulative exposure to a substance which forms such adducts. The concept of using Hgb adducts as a measure of internal dose has been extensively reviewed (Ehrenberg and Osterman-Golkar, 1980; Farmer *et al*, 1987; ECETOC, 1989). A variety of amino acid residues in Hgb (e.g., cysteine, histidine, N-terminal valine and C-terminal carboxylic acid) have been shown to be modified by many different chemicals.

There have been several studies related to styrene Hgb binding potential including *in vitro* studies, as well *in vivo* studies with both animals and humans. *in vitro* studies by Hemminki (1983) demonstrated that styrene oxide (SO) can react with a number of different polyamino acids, with polycysteine and polyhistidine having the greatest propensity for binding. Subsequent studies confirmed that SO binds to free amino acids *in vitro* with an affinity: cysteine > histidine > lysine > serine (Hemminki, 1986). Consistent with this observation, *in vitro* experiments with erythrocytes also showed the predominance of cysteine derivatives. A report by Kaur *et al* (1989), which also focused on *in vitro* binding of SO with amino acids or erythrocytes, indicated that there were modifications of histidine as well as cysteine in erythrocytes.

Byfalt-Nordquist *et al* (1985) reported the presence of an N-terminal valine adduct in Hgb of mice treated by i.p. injection with radiolabelled styrene (0.12 or 4.9 mmole/kg) or SO (0.063 or 1.1 mmole/kg). Osterman-Golkar (1992) also reported N-terminal valine adducts in Hgb from rats and mice given radiolabelled styrene or SO by i.p. injection at doses up to approximately 250 mg/kg. Attempts to measure binding to carboxyl groups in Hgb indicated that these adducts were unstable and unsuitable for quantitation. In contrast, Sepai *et al* (1992) recently reported a technique for monitoring exposure to SO by GC-MS analysis of phenylhydroxyethyl esters in Hgb. The studies by Osterman-Golkar showed that there was a disproportionate increase in Hgb adduct formation at i.p. doses of SO greater than 50 mg/kg; this was more evident in mice than in rats. Quantitative comparisons between the species showed that the formation of N-terminal valine adducts is approximately 3 times greater in mice than in rats given low i.p. doses of styrene. Both of these observations are consistent with interspecies differences in SO toxicokinetics: mice develop higher SO blood levels than rats, and SO blood concentrations become disproportionately higher in mice (but not rats) with increasing styrene doses (see Toxicokinetics section).

There have been a number of attempts to detect Hgb adducts in humans exposed occupationally to styrene. In a preliminary study, Sepai *et al* (1992) analysed samples from workers exposed to styrene, and found no evidence of adduct formation at a detection limit of 15 pmole/g globin. It was concluded that the difficulty in detecting adducts was associated with low binding efficiency of SO to globin. In a field study of 52 workers (styrene exposure 0 to 25 ppm) Severi *et al* (1993) found no evidence of styrene haemoglobin adduct formation

at a detection limit of 10 pmole/g globin and Farmer *et al* (1993), in a more limited study, also found no adduct formation at a similar limit of detection. Both investigators were however able to detect ethylene oxide adducts resulting from the endogenous production of ethylene. While Brenner *et al* (1991) failed to find a difference between styrene haemoglobin adducts in workers and controls, Christakopoulos *et al* (1993) reported a mean styrene haemoglobin adduct level of 28 pmole/g globin in 17 workers with a styrene exposure level of about 75 ppm as compared to a level of 10 pmole/g globin in non-exposed controls. The adduct level in the control (non-exposed) group was remarkably high and, as stated by the authors, may be an artefact. Ignoring the high background (control) level the mean adduct level of 28 pmole/g globin at an exposure level of 75 ppm is at about the same level of ethylene haemoglobin adduct formation resulting from endogenous ethylene production i.e. 8 to 25 pmole/g globin (Ehrenberg and Törnqvist 1992). At a styrene workplace exposure level of 20 ppm - at present recommended by a number of countries - styrene haemoglobin adduct formation has not yet been detected but can be expected to be lower than the unavoidable binding resulting from endogenous production of chemicals such as ethylene.

In addition to haemoglobin binding it has been suggested that DNA adduct formation, characterised by ^{32}P post-labelling techniques, could prove to be a useful method for biomonitoring human exposure (Vodicka *et al*, 1993). *in vitro* studies (Savela *et al* 1986 and Vodicka and Hemminki 1988) have indicated that SO can form covalently bound adducts with nucleophilic sites in isolated DNA *in vitro*. The major reaction site of SO in these studies was reported to be the N^7 position of guanine, although N^2 and O^6 alkylation products were also detected. More adducts were formed in single strand DNA than with double stranded DNA. Using the ^{32}P post-labelling technique, Pongracz *et al* (1992) reported that N^2-guanine derivatives were the major products of the reaction of isolated DNA with SO *in vitro*. Other investigations (Drinkwater *et al*, 1978) with isolated DNA have however failed to show an interaction of SO with DNA.

As part of a research programme on biomonitoring of human populations exposed to genotoxic environmental chemicals sponsored by The Commission of the European Communities (Directorate-General for Science, Research and Development) a number of studies were undertaken by Autrup (1992) to evaluate the potential for styrene DNA binding in laboratory animals, primarily using the ^{32}P post-labelling technique. Rats were exposed to styrene vapour concentrations of 0, 25, 150, and 300 ppm for 8 h. Immediately after cessation of exposure, one third of the animals were killed and the remaining animals were retained for a further 8 or 16 h. In a second study, mice were given a single intragastric dose of styrene at doses of 0. 0.001, 0.1, 1.0, or 10 mg/kg. The mice were killed 24 h after treatment. For the inhalation study, DNA from the following tissues were extracted and subjected to post-labelling analysis: brain, liver, colon, kidney, bladder, lung, femur, peripheral blood lymphocytes, bronchiae and nasal tissue. For the oral studies, all of the above tissues were examined with the exception of lymphocytes, bronchiae and nasal tissues. No adducts could be detected in any of the tissues that were evaluated.

In order to further investigate the potential for DNA adducts, rats were partially hepatectomized and treated with radiolabelled (^{14}C) guanine and (^{3}H) SO. The animals were placed in metabolism cages and 24 h urine samples were collected over a period of 3 d.

The urine samples with the highest radioactivity were eluted through a seppak column using a gradient of methanol as the solvent. The fractions were dried and further purified on a reverse phase HPLC column, and the double labelled fractions were separated and collected using a stepwise methanol/ammonium formate gradient. Analysis of the fractions with fast atom bombardment mass spectrometry showed no peaks at the expected molecular weights for styrene-guanine repair products, indicating that styrene-N7-guanine adducts were not excreted in the urine. A number of peaks with molecular weights different from styrene-guanine adducts were observed; the nature of these were not examined. (All results associated with these EC-sponsored studies have been forwarded to the Commission of the European Communities, Directorate-General for Science, Research and Development).

In addition to the study described by Autrup (1992) Latriano *et al* (1991) also used ^{32}P-postlabelling methodology in an attempt to detect SO-DNA adduct formation in experimental rats exposed to an atmosphere containing approximately 1,000 ppm of styrene, treatments lasting for 6 h/d for 5 d. The pattern of ^{32}P-postlabelling of lung, liver or lymphocyte DNA isolated from rats exposed to styrene was the same as that from the controls.

Cantoreggi *et al* (1993) used the ^{32}P-postlabelling technique to explore the possibility of DNA-adduct formation in the liver of mice exposed to styrene. Once again, even at a detection limit of 1 adduct/10^7 nucleotides, it was found impossible to detect, with any certainty, SO-DNA adduct formation.

There have been a number of reports describing the use of the ^{32}P-postlabelling technique to explore the possibility of DNA-adduct formation in exposed workers. Two papers, from the same laboratory, published in conference proceedings (Liu *et al*, 1988 and Boodle *et al*, 1990) reported studies on the analysis, after ^{32}P-postlabelling, of DNA isolated from the lymphocytes of styrene exposed worker(s). In the first report (Liu *et al*, 1988) DNA from a single exposed worker was compared with a single non-exposed control. In the second report (Bodell *et al*, 1990) a single result is again reported; although it is not stated whether both reports are results obtained from the same individual or even the same sample. The authors however claim to have observed evidence for the presence of SO derived DNA adducts in the exposed worker(s). The report appears to have a number of flaws. Firstly the numbers of individuals are far too small to provide any meaningful data. Secondly the fact that adducts seen in human DNA have similar chromatographic properties to those formed *in vitro* by SO must be treated with caution considering that the thin layer chromatographic system is a low resolution procedure. Thirdly and perhaps most significantly the adducts in the exposed worker(s) apparently co-migrate with the adducts that Bodell *et al* (1990) propose are bis-SO-desoxyguanosine (Pongrancz *et al*, 1992) and as stated above the formation of such adducts *in vivo* is extremely improbable. It is thus either a remarkable coincidence that DNA from the exposed worker(s) contain adducts that comigrate with SO-DNA adducts, or perhaps accidental cross-contamination of samples may have occurred. It is especially worth noting that to date there has apparently been no follow up on these studies and that neither report has appeared in a peer-reviewed journal.

Autrup (1992) conducted an analysis by ^{32}P-postlabelling of lymphocyte DNA isolated from 10 styrene exposed workers but failed to find any evidence for the presence of styrene-related

adducts. This result is perhaps not surprising since the same group failed to detect adducts in mice exposed to very high levels of styrene and also because of the low efficiency of the SO adducts generated *in vitro*.

In a report from Vodicka *et al* (1993) the authors described the reproducible measurement of specific O^6-adducts in DNA isolated from the leukocytes of workers exposed to high levels of styrene. This study could have important implications for biomonitoring and further studies are being planned to expand this work.

A major problem with using highly sensitive methods such as postlabelling is that there are many possible explanations for the presence of endogenous adducts (Philips *et al*, 1986; Reddy *et al*, 1990), including smoking (Jahnke *et al*, 1990; Savela and Hemminki, 1991) as well as diet-related adducts (Rothman *et al*, 1990). Inter-individual variations must also be taken into account. In an examination of the evidence for DNA and protein binding by styrene and styrene oxide (Philips and Farmer, 1994) the authors conclude that it is still difficult to make predictions about the applicability of ^{32}P post-labelling for biomonitoring of human exposure to styrene and that further study is required. They also conclude that styrene, via the production of styrene-7,8-oxide is not an effective alkylator and that the sensitivity of methods need to be enhanced for more widespread use.

In summary, SO has been shown to bind with amino acids, Hgb and DNA under *in vitro* conditions. Binding of SO to N-terminal valine in Hgb has also been shown in laboratory animals following exposure to high doses of SO or styrene. With further refinement and verification, measurement of Hgb adducts may possibly provide a useful biomonitoring method for styrene. Further work is still necessary (Philips and Farmer, 1994) before it will be possible to provide any useful assessment of the utility of the postlabelling assay for biomonitoring.

7.2 TOXICODYNAMICS

This section describes the toxicodynamics of styrene in experimental animals and man.

7.2.1 Animal Studies

7.2.1.1 Single Dose - Inhalation

Styrene has low acute inhalation toxicity. The acute inhalation LC_{50} for styrene in rats has been reported to be 2,700 ppm or 2,770 ppm for 4 h (Jaeger *et al*, 1974; Shugaev, 1969) and 4,620 ppm for 6 h (Bonnet *et al*, 1982). Spencer *et al* (1942) found that there was 100% mortality following an 8-h exposure to 5,000 ppm or a 3-h exposure to 10,000 ppm in both rats and guinea pigs, with an estimated 4-h LC_{50} of 6,000 ppm for rats and 5,200 ppm for guinea pigs. For mice, the 2-h LC_{50} has been reported to be 4,940 ppm (Shugaev, 1969) and the 6-h LC_{50} has been reported to be 2,430 ppm (Bonnet *et al*, 1982).

As can be expected from excessive exposure to any organic solvent, high concentrations of styrene can cause central nervous system depression. For example, single exposures of rats

and guinea pigs to 1,300 ppm styrene (12-30 h) resulted in weakness and unsteadiness, while 2,500 ppm for 10 h caused unconsciousness in both species (Spencer *et al*, 1942). Upon cessation of exposure, there was apparently full recovery from the acute central nervous system effects, with no permanent or irreversible effects.

7.2.1.2 Single Dose - Oral

The acute oral LD_{50} for rats has been reported to be approximately 5,000 mg/kg (Wolf *et al*, 1956); another report (Hinz *et al*, 1980) gives acute oral LD_{50} values of 8,060 mg/kg (77.4 mmol/kg) for male and 6,650 mg/kg (63.9 mmol/kg) for female rats. However, repeated oral dosing of animals at 2,000 mg/kg was highly irritating to the esophagus and stomach, resulting in the death of animals after only a few doses (Spencer *et al*, 1942). The oral LD_{50} in mice has been reported as 316 mg/kg (Litton Bionetics, 1973).

7.2.1.3 Single Dose - Dermal

A dermal LD_{50} has not been determined; there are no data that show styrene to be absorbed through the skin in acutely lethal amounts.

7.2.1.4 Single Dose and Short Term - Irritation

Styrene is irritating to the skin, eyes and respiratory tract. Styrene has been reported to be a slight to moderate skin irritant based on a single exposure in rabbits (Wolf *et al*, 1956; Shugaev, 1969; Hatoum and Johnson, 1991a). Repeated or prolonged skin contact can cause defatting and dehydration of the skin leading to dermatitis (Spencer *et al*, 1942). In a recent acute eye irritation study in rabbits, moderate eye irritation with no corneal opacity observed at 1 h following administration of styrene decreased to slight irritation at 72 h and was no longer present at 21 d (Hatoum and Johnson, 1991b). A previous study had reported moderate conjunctival irritation and transient corneal injury of the eyes when undiluted styrene was tested in rabbits (Wolf *et al*, 1956). High vapour concentrations in the range of 650-1300 ppm caused immediate signs of eye and nasal irritation in rats and guinea pigs (Spencer *et al*, 1942). Alarie (1973) investigated the sensory irritation potential of styrene (and other chemicals) in mice. The concentration of styrene which results in a 50% decrease in the respiratory rate (RD_{50}) in mice exposed for three minutes was reported to be approximately 160 ppm. The sensory irritation was thought to occur via reaction of styrene (or metabolites) with sulfhydryl groups on the free afferent trigeminal nerve endings located on the surface of the nasal mucosa.

7.2.1.5 Sensitization

There is no evidence from all of the many studies in experimental animals and humans that styrene produces skin or respiratory sensitization in exposed populations.

7.2.1.6 Repeat Dose - Inhalation

There have been a number of studies to investigate the effects of repeated exposure to styrene in animals, but many of these studies have focused only on certain selected aspects of toxicity (e.g., liver enzymes) rather than on a general overall assessment. In one of the older studies with limited histopathologic examinations, no toxicologic effects were noted when small groups of rabbits and primates were exposed via inhalation to about 1450 ppm of styrene, 7 h/d, 5 d/wk, for approximately 6 months (Spencer *et al*, 1942). Rats subjected to similar exposures showed signs of irritant effects on the eyes and nasal mucosa, while approximately 10% of the guinea pigs exposed to 1450 ppm died during the course of the first few exposures. Autopsy of these guinea pigs revealed signs of severe irritant effects in the lungs; guinea pigs that survived the 6-month exposure period had a marked reduction in weight gain, and appeared to be in poor condition.

No effects were noted in either rats or guinea pigs exposed to 650 ppm styrene 7 h/d, 5 d/wk, for approximately six months (Wolf *et al*, 1956). At 1300 ppm, there were indications of eye and nasal mucosa irritation in both species, as well as reduced weight gain for the guinea pigs. Reduced weight gain and irritant effects on the eye and nasal mucosa were noted for both species at 2,000 ppm. At autopsy, no lesions were noted in either species at any dose level, in any of several major organs and tissues that were evaluated. Likewise, Wolf *et al* (1956), using a similar exposure regimen as for rats and guinea pigs, found no effects in small numbers of rabbits exposed to 2,000 ppm styrene, or in rhesus monkeys exposed to 1300 ppm.

A study focused on the irritant effects of styrene vapours on the respiratory tract revealed morphologic changes (vacuolation of epithelial cells, nuclear pyknosis, and "fall-off" of epithelial cells) in the nose and trachea of rats exposed to 800 ppm styrene 4 h/d for 8 wk (Ohashi *et al*, 1985). Additional studies by Ohashi *et al* (1986) indicated that there was a dose-dependent decrease in tracheal and nasal ciliary activity in rats exposed to 150 or 1,000 ppm styrene for 3 wk. There was nearly complete recovery by the 12th wk after the last exposure to 150 ppm, and at least partial recovery was observed in animals exposed to 1,000 ppm.

Vainio *et al* (1979) reported minor histological liver alterations (parenchymal hydropic degeneration, steatosis, and congestion), but no lung or kidney effects in rats exposed to 300 ppm styrene 6 h/d, 5 d/wk, for up to 11 wk. Hepatic glutathione (GSH) levels were significantly decreased after 2 wk; thereafter the depression in hepatic GSH levels was less pronounced but still evident throughout the 11-wk exposure period. Lung GSH levels were also significantly decreased after 2-wk and 4-wk exposure to 300 ppm, but when measured after 6 or 11-wk of exposure the lung GSH levels were slightly higher than for controls. Additional studies indicated that hepatic GSH levels were also depressed in rats when measured 30 minutes after exposure to 200 ppm or 400 ppm styrene vapours, 6 h/d, for 4 d. The decreases in hepatic GSH levels were reversible, since by 18 h after the exposure the liver GSH levels of treated animals were even higher than for controls. Elovaara *et al* (1990) reported a 43% decrease in pulmonary non-protein sulfhydryls (NPSH) and a 24% decrease in liver NPSH in rats exposed to 500 ppm styrene, 5 h/d, for 3 d. A single 24 h exposure to

500 ppm likewise resulted in a substantially larger decrease in pulmonary NPSH (66%) as compared to the liver (16%).

Liver, but not kidney, GSH levels were significantly depressed in male Wistar rats after 4, 8, 13, and 17 wk (6 h/d, 5 d/wk) of exposure to 300 ppm styrene by inhalation (Elovaara *et al*, 1979). Co-administration of ethanol as a 15% solution in drinking water was considered by the authors to have more than an additive effect on the hepatic GSH depletion.

Studies by Vainio and Makinen (1977) with mice, rats, hamsters, and guinea pigs indicated that the mouse was most vulnerable and the rat most resistant to styrene-induced toxicity. This observation has been supported by inhalation studies designed to provide an overall assessment of the toxicologic potential of styrene in male and female rats and mice prior to the initiation of carcinogenicity studies. The results of these studies show a pronounced species difference in the sensitivity of Fischer 344 rats and B6C3F1 mice, as well as a sex difference in the sensitivity of mice (Roycroft *et al*, 1992, Morgan *et al*, 1993). In the investigation with Fischer 344 rats the animals survived exposure to styrene concentrations of 500, 1,000 or 1500 ppm (6 h/d, 5 d/wk) for 90-d with no overt indication of toxicity except for minor exposure related histopathological changes in the olfactory epithelium seen at all dose levels. Styrene did not affect cell replication in liver or lung at any exposure level (Cruzan *et al*, 1993). In comparison when B6C3F1 or CD1 mice were exposed to 250 or 500 ppm styrene for 6 h/d for up to 14 d toxic effects characterised by deaths, hepatotoxicity and lung toxicity were observed (Cushman *et al* 1993). A marked sex difference in toxicity was also seen with the percentage of mortalities of male mice being substantially higher than for females. This was accompanied by a peculiar inverse dose-response relationship in that the incidence of mortalities was higher in the 250 ppm treatment group than in the 500 ppm group (Morgan *et al*, 1993 and Cushman *et al* 1993). Additional studies have also shown a clear strain difference in the sensitivity of B6C3F1, C57bl, CD-1 and DBA mice exposed to 250 or 500 ppm styrene, 6 h/d, for 14 d (Morgan *et al*, 1992; Mahler *et al*,1992). For males, B6C3F1 mice were the most sensitive strain, with a high incidence of mortality occurring at 250 ppm; the inverse dose-response relationship was again apparent since fewer mortalities occurred at 500 ppm than at 250 ppm. DBA mice were the most resistant, with no mortalities occurring at either 250 or 500 ppm. CD-1 and C57bl male mice were of intermediate sensitivity. For females, CD-1 mice were the most sensitive strain, with a slightly higher incidence of mortality at 250 ppm than at 500 ppm. There was a very low incidence of mortalities for both B6C3F1 and C57bl female mice.

Substantially different results were obtained in another study involving only the B6C3F1 and CD-1 strains of mice (Coombs, 1992). In this investigation no clear strain differences were observed. However, females of both strains had higher mortalities at 250 ppm than at 500 ppm, while males of both strains had higher mortalities at 500 ppm than at 250 ppm. Additional studies are currently in progress to develop a more thorough understanding of the species and strain differences and to determine which strain of mice, if any, is most relevant for a chronic inhalation bioassay.

Vainio and Makinen (1977) have suggested that differences in susceptibility to styrene toxicity exhibited by the mouse and rat may be explained by changes in hepatic NPSH content. This

proposition was based on data showing that one hour after receiving a 300 mg/kg ip dose of styrene in olive oil, the hepatic NPSH content was depleted 60% in mice while "only a small decrease" in hepatic NPSH content was measured in the rat. The authors suggested that the species differences in NPSH depression may be due to differences in the rates of formation and degradation of an active intermediate (e.g. styrene oxide), since the mouse, in comparison with the rat, has a rather high epoxide-forming and low epoxide-inactivating activity. Studies by Morgan *et al* (1992) have however shown that the higher incidence in mortality seen in male B6C3F1 mice, as compared with females receiving similar treatments (i.e.250 ppm or 500 ppm styrene vapours for 3 d) cannot be explained by more extensive hepatotoxicity or greater GSH depletion in the males.

7.2.1.7 Repeat Dose - Oral

Repeated doses of 1,000 mg/kg to rats or 250 mg/kg to mice resulted in increased mortality; signs of toxicity in animals that died included marked irritant effects on the oesophagus and stomach (NCI, 1979). In a study reported by Wolf *et al* (1956), repeated doses of 400 mg/kg in olive oil for six months resulted in decreased bw gain, and increased liver and kidney weights with no associated histological changes; the no effect level in these studies was about 130 mg/kg.

Das *et al* (1981) reported a dose-related decrease in hepatic GSH in rats given 270, 450 or 900 mg/kg for seven d. The same researchers dosed male albino rats with 200 or 400 mg/kg styrene per d, 6 d/wk, for 100 d (Srivastava *et al*, 1982). They reported that while there was no overt toxicity or changes in bw or liver weight, focal areas of hepatic necrosis and increases in serum GOT and GPT were observed in the 400 mg/kg dose group, but not in the 200 mg/kg group. An additional study by Srivastava *et al* (1989) suggested an effect of oral administration of 400 mg/kg/d styrene on testicular function and morphology of rats, but this report should be considered preliminary because of several deficiencies including small numbers of animals and questions about purity of the test material. Numerous other subchronic and chronic styrene studies have shown no testicular effects related to styrene.

A decrease in renal glutathione content and decreased glutathione-S-transferase activity has been reported in rats treated with styrene at a dose of 900 mg/kg for 7 d (Das *et al* 1983). Wolf *et al* (1956) reported an increase in kidney weights in female rats administered styrene for 6 months at doses of 400 or 667 mg/kg/d. Treatment was not associated with any histopathological changes. There are reports indicating that conjugation of styrene oxide with glutathione gives rise to mercapturates which may have nephrotoxic properties under certain conditions, apparently via an inhibition of the renal organic anion transport system (Craan and Malick, 1989; Chakrabarti and Malick, 1991). However, these studies involved either *in vitro* approaches or high intravenous doses of the glutathione conjugate, and the relevance to styrene exposures *in vivo* is uncertain.

Male and female purebread beagle dogs were exposed to 0, 200, 400 or 600 mg/kg bw/d by gavage for up to 561 d (Quast *et al* 1979). Treatment in the high dose (600mg/kg/d) group was stopped on day 316 and resumed on day 470 for a further 90 d to study reversibility of effects. There were only very minimal toxicological changes; the most prominent being intra-

erythrocyte Heinz bodies present, in a dose related manner in males and females in the 400 and 600 mg/kg dose groups. The formation of the Heinz bodies was reversible on discontinuing treatment in the 600 mg/kg dose group.

7.2.2 Human Studies

Human volunteers exposed to 800 ppm experienced eye and throat irritation, runny nose, metallic taste, as well as drowsiness and vertigo in a 4-h exposure (Carpenter *et al*, 1944).

At 600 ppm, the odour of styrene was reported to be very strong, and human volunteers experienced eye and nasal irritation. The odour of styrene was described as objectionably strong at 200-400 ppm, strong but tolerated without excessive discomfort at 100 ppm, and detectable but non-irritant at 60 ppm (Wolf *et al*, 1956). In another report, concentrations of 376 ppm for one hour caused eye and nasal irritation in human volunteers, while 216 ppm for one hour caused only nasal irritation in one volunteer (Stewart *et al*, 1968). Volunteers exposed to 99 ppm for 7 h had mild transient eye or throat irritation. There were no exposure-related findings in volunteers exposed to 51 ppm for one hour or 117 ppm for two h. The odour perception threshold for styrene in air has been reported by Smith and Hochstettler (1969) to be 0.05 to 0.08 ppm while Hellman and Small (1974) reported an odour detection threshold of about 0.02 ppm. In addition to the symptoms of irritation there is evidence that styrene exposures may affect the central nervous system - this is discussed in detail in Section 7.2.10.

Studies examining the effect of styrene exposure on renal toxicity have in the main been restricted to a few studies involving urinalysis. In a cytological examination of urinary deposits from laminate workers exposed to styrene for periods ranging from 0.5 to 14 years Härkönen (1977) failed to find any changes associated with exposure. Askergren *et al* (1981a) in a study of workers exposed to organic solvents, especially to styrene, 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 39 unexposed controls. While no changes in glomerular filtration rates were observed, a greater number of cells were found in the urine of exposed workers as compared with controls. In the same study the authors reported that workers exposed to organic solvents excreted significantly larger quantities of urinary albumin than unexposed workers while no differences were observed in β-2-microglobulin excretion. In other studies researchers have used the measurement of urinary enzymes, e.g. alanine amino peptidase (AAP), β -galactosidase (GAL), β-glucuronidase (β-GLU) and N-acetyl -β-D-glucosaminidase (NAG), and/or urinary proteins e.g. retinol binding protein (RBP), albumin (ALB) and β2-microglobulin (β2M) as markers for early effects on glomerular and tubular function. For example Franchini *et al* (1985) and Vyskocli *et al* (1989) reported, based on measurements of urinary β-GLU and NAG activities, a lack of nephrotoxicity at an average styrene exposure level of 50 ppm. In contrast after measuring AAP, GAL, and NAG activities, and RBP levels in the urine of 10 styrene exposed workers (exposure 5 to 94 ppm) and 15 non-exposed individuals Verplanke and Herber (1992) concluded that styrene exposure may have an effect on tubular and glomerular function. Two other studies measuring similar parameters i.e. Lauwerys and Bernard, (1985) and Viau *et al* (1987) failed to find evidence suggesting styrene exposure may produce toxic kidney effects.

No significant difference in glomerular filtration rate (measured by clearance of ^{51}Cr-EDTA) was observed between 33 styrene workers and 48 controls (Askergren *et al* 1981b).

There have been a number of investigations to examine if occupational exposure to styrene is associated with hepatic effects especially induction of toxicity. A health survey of a small group of Dutch workers processing reinforced plastics mainly in boat making was carried out by Zielhuis *et al* (1963). One group of workers, comprising 29 men had average exposure over the workshift estimated to be in the range 24 to 94 ppm but may have had exposed to mean concentrations in the range 106 to 209 ppm while working in partially closed boats peak exposures were estimated to be 235 to 705 ppm. A second group of 28 workers had lower exposures ranging from 14 to 74 ppm. Each of the workers was subjected to a physical examination, blood tests (including haematology, bilirubin and serum proteins) and urine analysis. No significant difference was found when the results of the exposed group were compared with data from a control group of 31 individuals. A similar health survey of 526 individuals exposed to styrene in a Russian factory manufacturing divinyl rubber (Kats 1962) reported increased incidence of hepatomegaly (30% of workforce) impaired liver function as indicated by reduced serum albumin and raised serum beta and gamma-globulin levels. The value of both studies is limited; the first by the small numbers of individuals and the second by the lack of detail especially about exposure levels and the "changes" observed.

The health status of 488 styrene-polystyrene polymerisation workers, including an evaluation of hepatic effects, has been described by Lorimer *et al* (1978). Blood samples collected from the workers, classified into high (288 individuals) and low (200 individuals) exposure groups, were analysed for serum enzyme activities including alkaline phosphatase (AP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and gamma glutamyltranspeptidase (gamma-GT). The results from each sub-group was compared to those from 993 men tested in the same laboratory. The only difference noted was the significantly elevated prevalence of abnormal gamma-GT values seen in the high exposure group i.e. 7% of the high sub-group had elevated serum gamma-GT activities as compared to 2% found in the low-dose group and controls. The authors suggest that slight increase in gamma-GT activity could be associated with enzyme induction related to styrene metabolism rather than release of enzymes resulting from cytotoxicity.

A number of other studies have also used serum enzyme measurements as markers for liver dysfunction. For example Axelson and Gustavson (1978) measured serum AP, ASAT, ALAT activities in blood samples from 35 styrene-exposed male workers (TWA about 100 ppm) and 12 unexposed controls. Although the average transferase levels were slightly higher among the exposed group only ASAT was significantly elevated ($P < 0.001$). No differences were found in the average levels of AP. The effect of styrene on plasma activities of ALAT, ASAT, gamma-GT and ornithine carbonyl transferase in workers exposed to different concentrations of styrene i.e. high exposure (50 to 100 ppm) and low exposure (<50 ppm), was examined by Hotz *et al* (1980). The results showed that the distribution of enzyme activities shifted towards higher values from the low to the high exposure groups. The majority of the activities were however within the clinically normal range.

Lundberg (1981) measured serum levels of ALAT, ASAT, gamma-GT, LDH, and SDH (sorbitol dehydrogenase) in 72 workers designated as either having low (<5 ppm) or high (41 ppm) exposures to styrene; but although the styrene-exposed groups had a higher mean gamma-GT value than the control group ($P < 0.005$) the effect was not dose related. Vihko *et al* (1983) studied a variety of parameters (i.e. serum activities of ALAT, ASAT, gamma-GT, 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) in a group of 25 individuals exposed to styrene at about 30 to 40 ppm. The most frequent observation apparently related to styrene exposure was an elevated concentration of chenodeoxycholic acid. Spasovski (1976) reported changes in serum protein profiles and elevated serum transaminase activity in workers exposed to styrene at concentrations of 500 ppm. No differences in clinical chemistry and haematological parameters were seen in 98 men exposed to styrene in the Finnish polyester resin industry as compared with a control group of men not exposed to styrene (Seppalainen and Harkonen, 1976). Similarly when Harkonen *et al* (1984) measured serum enzyme activities and cholic acid and deoxycholic in 34 styrene-exposed and 34 reference workers no difference could be found between the groups.

The cardiovascular and respiratory systems have been the subject of a limited number of studies. A comparison of thorax radiographs of 84 workers taken before the beginning of employment and after exposure to styrene a (50-300 ppm) for 1-36 years indicated no grossly observable changes attributable to styrene exposure (Thiess and Friedheim, 1978). The authors could also find no evidence of gross pathological indications in electrocardiograms of exposed workers compared with those of a reference group of 62 subjects. Götell *et al* (1972) reported that lung function tests (forced vital capacity and forced expiratory volume in 1 second) measured in 15 workers (TWA styrene concentration 17-290 ppm) were normal and did not change during the working day. In a cross-sectional study of 488 styrene production-manufacturing workers, classified as having low and high styrene exposure, Lorimer *et al* (1978) reported that individuals categorised as having high exposures (approximately 20 ppm) described themselves as having a greater incidence of respiratory infections as compared with the group categorised as having low exposures (approximately 1 ppm). Indices of obstructive, restrictive and small airway dysfunction were also studied but lack of comparisons with untreated control groups and possible co-exposures to benzene and butadiene makes interpretation of the data very difficult. Chest X-rays exhibited no noteworthy changes associated with styrene exposure. When 98 male laminate workers occupationally exposed to styrene, were examined for symptoms of simple chronic bronchitis (Härkönen, 1977) 28% of the exposed group appeared to have simple chronic bronchitis, compared with 12% in the control group. An examination of a subgroup (i.e. 43/98) of the workers showed that lung function tests and chest x-rays were normal with no signs of pneumoconiosis. Seppalainen and Harkonen (1976) performed lung function tests on 98 men exposed to styrene and compared the results with a similarly sized control group. No significant difference was noted between the control and styrene exposed populations.

Studies assessing the haematological effects of styrene on humans includes the investigation undertaken by Chmielewski and Renke (1976) on a group of 101 workers exposed for at least one year to a styrene concentration of 24-72 ppm. No significant effects were noted on the haemoglobin concentration, erythrocyte count, leucocyte count, or differential count. Some

workers exposed for more than 10 years, however, had a slightly decreased thrombocyte count compared with workers exposed for shorter periods. Those individuals with reduced platelet counts also showed increased coagulation times and platelet adhesivity although prothrombin ratio and fibrinolysis time were reduced. Lorimer *et al* (1976) in a study on 486 workers, with different levels and durations of styrene exposure, observed a random distribution of abnormal haemoglobin concentrations, and leucocyte or platelet counts between the groups. Thiess and Friedheim (1978), who investigated 84 workers exposed to 50-500 ppm for 1-36 years, did not notice any appreciable differences in haemoglobin concentrations, or leucocyte, erythrocyte, or platelet counts compared with a reference group. A cross-sectional survey on production workers at a US SBR manufacturing plant with relatively low exposures to styrene i.e. usually <1 ppm (in some areas exposures did reach about 14 ppm) found no evidence of haematological abnormalities (Checkoway and Williams, 1982).

Two reports have been published describing the measurement of the urinary excretion of 17-ketosteroids or 17-ketogenic steroids. In one investigation (Wink 1972), involving 25 workers, exposed to styrene, and 26 age-matched controls no significant differences in the urinary excretion of 17-ketosteroids or 17-ketogenic steroids was seen. However, Chmielewski *et al* (1973) reported reduced excretion of 17-ketosteroids in urine in 27 out of 67 workers exposed to styrene concentrations of 24 - 72 ppm.

7.2.3 Summary and Evaluation

The most prevalent and reproducible effect noted in experimental animals and workers occupationally exposed to styrene is irritation of eyes and nasal mucosa (CNS effects are discussed below). The results in general indicate that in humans mild irritant effects begin at about 100 ppm with more marked effects e.g. conjunctivitis, occurring at levels around 200 ppm. Irritation to the respiratory tract also occurs at these higher exposure levels although non of the studies have indicated a lasting effect of impairment to lung function.

The equivocal nature of the data regarding effects of styrene on hepatic and renal function make it impossible to exclude the possibility that exposure to high levels of styrene may produce mild hepatic and/or renal dysfunction. However the conclusion is justified that if slight changes in renal or hepatic functions do occur following high exposures to styrene such alterations have little or no clinical consequence, also in view of the years which styrene has been in commercial production, the numbers of people which have been exposed occupationally and the health records which are maintained for these populations.

7.3 GENOTOXICITY - CYTOGENETICS

7.3.1 Cytogenetics - Human Studies

A summary of the studies measuring chromosomal aberrations, SCE and/or micronuclei in styrene exposed workers is shown in Tables 11, 12 and 13 respectively. Of the approximately 30 chromosomal aberration studies half have no cytogenetic effects, 3 gave equivocal results and the others reported a positive correlation between styrene exposure and chromosomal aberrations (Table 11). Of the 11 "positive studies" 9 were conducted with populations below the minimun number of 25 exposed and 25 control individuals recommended by Ashby and Richardson (1985). Of the two remaining positive studies i.e. Camurri *et al* (1983 and, 1984) and Andersson *et al* (1980) only the latter can be regarded as being adequate in terms of the numbers of individuals examined i.e. 36 individuals in the exposed group and 37 in the control group. Although the studies by Camurri *et al* (1983 and 1984) included >25 individuals they were "pooled workers" from 9 plants in which there were different exposures to styrene (range about 7 - 96 ppm). The results of this study are rather strange in terms of dose response characteristics since, although the authors reported threefold increase in aberration yields at an average exposure of about 10 ppm, there was no further significant increase in aberration levels with increasing exposure concentrations i.e. levels of aberrations reported at 10 ppm exposure were the same as that seen in workers with an average exposure at 96 ppm. Camurri *et al*, also examined SCE (Table 12) and reported no significant increase in SCE until exposures exceeding 48 ppm at which point there was a very sharp increase in the frequency of SCE i.e. approximate doubling at 70 ppm. No further rises were seen however even in workers with styrene exposures >96 ppm. In addition to the lack of any dose - response relationships it is interesting to note that the increased SCE levels were seen in only 3 of the 9 plants thus it is possible that the effects were more related to other chemicals used at these 3 plants rather than to styrene used at all 9 locations.

In the study reported by Andersson *et al* (1980) a total of 36 persons were included in the "exposed" group; 22 in the low level group, and 14 in the high level group. The low exposure group had mean total exposures of 32 ppm (range 1 - 67 ppm); the high exposure group had mean total exposures of 283 ppm (range 167 - 374 ppm). The mean employment time for the low group was 3 years, and for the high group 7.6 years. The control group consisted of 37 persons who worked in offices, workshops and the assembly shop. There was a small increase in total aberrations in the exposed group compared to the control (7.9% vs. 3.2%), and this increase was largely due to chromatid and isochromatid deletions, the types of aberrations most frequently induced by the majority of chemical agents, including alkylating agents. There was no difference in aberration frequency between low and high exposure groups, although the authors claim a linear dose response within the low exposure. Observation of the data does not support this conclusion. The increase in aberrations observed in the exposed group cannot be deduced to be caused by styrene because there is no dose response relationship with styrene exposure, and because styrene is not the only agent in the workplace environment that has the potential to be clastogenic.

Table 11: Chromosome Aberrations in Lymphocytes of Workers Exposed to Styrene

Numbers studied		Years of exposure	Styrene conc in air (ppm)		Urinary MA		Culture time (h)	Chromosome aberrations		Result	Reference
Exposed	Controls		Mean	Range	Mean	Range		Exposed	Controls		
10	5	0.6-8.5	-	up to 300	721	23-3257	64-66	16.6	1.8	P	Meretoja *et al* (1977, 1978a)
15	6	1-15	-	up to 300	493	23-3257	64-68	14.4	2.0	P	"
5	20	14-25	-	up to 10	30	19-40	70-72	3.8	5.5	N	Fleig and Theiss (1978)
12	20	3-39	2	0-47	32	< 5-100	70-72	5.1	5.5	N	"
14	20	2-24	-	50-300	593	42->1500	70-72	9.2	5.5	P	"
6	6	0.5-10		14-192	490	225-2100	72	10.2	4.9	P	Hogstedt *et al* (1979)
24	24	4-27	-	0.7-178	-	0-320	70-72	5.1	3.8	N	Theiss *et al* (1980)
36	37	0.3-12	-	0-232	-	-	66-68	12.3	6.7	P	Andersson *et al* (1980)
16	13	0.6-9.3	-	1-211	-	90-4300	-	3.4	3.3	N	Watanabe *et al* (1981)
18	6	0.2-30	-	40-50	332	0-1041	50	6.5	4.7	N/P	Watanabe *et al* (1983)
30	2	1-30	<23	-	-	-	68	24.6	16.7	P	Dolmierski *et al* (1983)
24	21	1-22	-	7->96	458	45-1108	50	34.5	7.0	P	Camurri *et al* (1983, 1984)
41	30	1-22	-	7->96	479	45-1440	50	30.7	6.9	P	"
18	9	-	13.2	2-44	-	-	48-53	20.7	15.1	P	Hansteen *et al* (1984)
15	13	1-26	24	-	-	<152-304	64-68	2.8	2.7	N	Nordenson and Beckman (1984)

Numbers studied		Years of exposure	Styrene conc in air (ppm)		Urinary MA		Culture time (h)	Chromosome aberrations		Result	Reference
Exposed	Controls		Mean	Range	Mean	Range		Exposed	Controls		
105	136	-	0.01	<0.1-1.4	-	-	-	2.8	3.9	N	van Sittert and de Jong (1985)
36	19	1-11	-	1-236	-	35-972	54	3.4	2.9	N/P	Pohlova and Sram (1985)
22	22	1-11	-	9-132	-	40-3,000	54	5.3	3.7	N/P	"
21	21	1-25	24	8-63	243	0-1064	50	4.5	4.9	N	Maki-Paakkanen (1987)
32 8	32 8	18.8 (mean) 4.5 (mean)	- -	0.4-60 10-48	- -	- -	48 48	3.2 4.25	2.9 2.9	P N	Forni *et al* (1988)
11	11	10 (mean)	61	28-140	-	-	50-52	1.5	2.2	N	Jablonicka *et al* (1988)
11	14	0.1-25.4	1.3	1-38	-	-	48	1.9	3.2	N	Hagmar *et al* (1989)
11	11	6.4 (mean)	-	-	1679	up to 3,268	50	4.9	6.8	N	Maki-Paakkanen *et al* (1991)
6	6	7.2 (mean)	-	-	988	up to 2,523	50	6.0	3.7	N	"
17	17	6.7 (mean)	70	-	1429	up to 3,268	50	5.3	5.7	N	"
50	54	-	43	5-182	-	-	50	1.9	1.7	N	Sorsa *et al* (1991)
25	54	-	11	1-333	-	-	50	1.9	1.7	N	"
109	54	-	-	14-39	-	-	-	-	-	N	Norppa *et al* 1991)
7	7	1-18	-	5-24	-	46-345	48	1.6	1.4	N	Tomanin *et al* (1992)
11	11	2-20	-	26-102	-	423-1,325	48	3.8	0.8	P	Tomanin *et al* (1992)
18	18	5-22	-	-	328	-	-	5.1	2.2	P	Anwar and Shamy (1993)

Table 12: SCE in Lymphocytes of Workers Exposed to Styrene

Numbers studied		Years of exposure	Styrene concentration in air (ppm)		Urinary MA		Result		Reference
Exposed	Controls		Mean	Range	Mean	Range			
11	3	1-15	-	up to 300	721	23-3257		P	Meretoja *et al* (1977)
20	21	0.3-12	-	0-232	-	-	7.5	N	Andersson *et al* (1980)
16	13	0.6-9.3	-	1-211	-	90-4300	7.6	N	Watanabe *et al* (1981)
18	6	0.2-30	-	40-50	332	0-1041	8.5	N	Watanabe *et al* (1983)
22	20	1-22	-	7->96	445	45-1038	10.8	P	Camurri *et al* (1983, 1984)
35	28	1-22	-	7->96	480	45-1440	10.4	P	Camurri *et al* (1983, 1984)
18	9	-	13.2	2-44	-	-	6.5	N	Hansteen *et al* (1984)
21	21	1-25	24	8-63	243	0-1057	7.4	N	Maki-Paakkanen (1987)
7	8	mean=8.6	50	1.7-131	275	-	7.2	N	Kelsey *et al* (1990)
13	12	mean=7.2	55	5.8-130	323	-	6.2	N	"
11	11	mean=6.4	-	-	1679	up to 3268	12.9	N	Maki-Paakkanen *et al* (1991)
6	6	mean=7.2	-	-	988	up to 2523	11.4	N	"
17	17	mean=6.7	70	-	1429	up to 3268	12.4	N	"
10	9	mean=2.7	11.2	1-44	243	96-2495	10.1	N	Brenner *et al* (1991)
70	31	-	-	14-39	-	-	-	N	Norppa *et al* (1991)
50	54	-	43	5-182	-	-	7.1	N	Sorsa *et al* (1991)
25	54		11	1-133	-	-		N	"
25	54	-	7	0-25	98	8-504		N	Severi *et al* (1993)
46	-	0.5 - 27	15.4	02-56	-	-	7.1	P	Yager wt al (1993)

Table 13: Micronuclei in Lymphocytes of Workers Exposed to Styrene

Numbers studied		Years of Exposure	Styrene conc. in air (ppm)		Urinary MA		MN per 10^3 cells		Result	Reference
Exposed	Controls				Mean	Range	Exposed	Controls		
0	5	0.6-8.5	-	up to 300	721	23-3,257	8.8	0.8	P	Meretoja *et al* (1977)
38	20	1-23	13	1-40	65	9-316	5.9	3.6	P	Hogstedt *et al* (1983)
12	12	1-26	24	-	-	<152-304	3.5	0.8	P	Nordenson and Beckman (1987)
21	21	1-25	24	8-63[9]	243	0-1,064	1.5	1.6	N	Maki-Paakkanen (1987)
20	22	0.1-25.4	13	1-32	-	-	4.3	4.4	N	Hagmar *et al* (1989)
10	9	mean=2.7	11.2	1-44	243	96-2,495	10.3	6.5	P	Brenner *et al* (1991)
11	11	mean=6.4	-	-	1679	up to 3,268	13	11	N	Maki-Paakkanen *et al* (1991)
6	6	mean=7.2	-	-	988	up to 2,523	15	14	N	"
17	17	mean=6.7	70	-	1429	up to 3,268	14	12	N	"
50	54	-	43	5-182	-	-	7	8	N	Sorsa *et al* (1991)
25	54	-	11	1-133	-	-	7	8	N	"
50	37	-	-	17-39	-	-	-	-	N	Norppa *et al* (1991)
7	7	1-18	-	5-24	-	46-345	8.7	10.2	N	Tomanin *et al* (1992)
11	11	2-20	-	26-102	-	423-1,325	12.6	8.5	N	"
46	-	0.5-27	15.4	0.2-56	-	-	8.9	-	N	Yager *et al* (1993)
52		-	7	0-25	98	8-504	-	-	N	Severi *et al* (1993)

N = negative result P = positive result

The authors also reported a slight increase in SCE in the exposed group compared to the control group. However, the difference was not significant and there were concomitant exposures to other chemicals. In addition, the SCE frequency was lower in exposed smokers vs. non-smokers. It is reasonable to conclude that styrene exposure did not increase the SCE frequency.

The remaining 9 positive chromosomal aberration studies have, in addition to the low numbers used, a variety of other problems. The study by Meretoja *et al* (1977), which included only ten persons employed in three different plants, the lymphocytes were cultured in TC199 which because of the low folate level is inappropriate for culturing human lymphocytes (i.e. background frequency of chromosomal aberrations is higher when lymphocytes are grown in culture medium containing low folate concentrations - see Preston ,1990). A further interesting point with this report is that while a diagram in the paper showed a cell with an aberration known as a dicentric no dicentrics are recorded in the results. Although the results indicate an increase in "chromosome breaks" in the styrene group there is no relationship of aberration frequency to estimated exposure, and information on other chemicals present in the workplace is not provided. These factors, together with the fact that the sample sizes are very small, and the aberration type observed is difficult to explain, means that this study cannot be considered to be informative with regard to the effects of styrene on aberration induction in lymphocytes.

The study of Meretoja *et al* (1978b) represents a restudy of the same persons reported in Meretoja *et al* (1977) with once again the authors reporting an increase of total aberrant cells in the test group as compared to the control. This restudy has however in addition to the technical problems described for the original investigation other limitations including the fact that gaps were included in the category of aberrations and the control group was not resampled - the aberrant cell frequency simply being the one obtained one year previously. As a result no conclusions on the clastogenicity of styrene can be drawn from these studies. The study also reports on the analysis of SCE in 11 persons employed in the reinforced plastics industry, and 3 control persons. Cells were cultured for 66-68 h and 2nd division metaphases analysed. There was no difference in SCE frequency between exposed and control groups which contrasts with the reported increase in aberrations.

Hogstedt *et al* (1979) conducted a small study on six persons employed for 0.5 to 10 years in the manufacture of fibreglass-reinforced polyester resin boats. The frequency of aberrations was measured and compared to a control group of 6 persons. While the authors reported an increased frequency of aberrations in the test group compared to the control there was however an overlap in frequencies between individuals in the two groups and the difference in means for "breaks" was very small. Even if the difference was significant because the workers were probably exposed to a range of other agents, which may or may not be potential clastogens, the increase clearly cannot be attributed specifically to styrene exposure. In fact, no definite conclusions can be drawn from this study.

The same types of criticisms can be levelled at all of the other "positive" studies and for a in-depth evaluation the reader is recommended to see Preston (1990).

While the positive results are fraught with problems many of the negative reports can also be criticised for including populations below the 25 recommended by Ashby and Richardson (1985). There are however a number of studies in the negative reports which do include adequate population sizes and are not subject to some of the technical problems described above. For example Norppa *et al* (1991) reported that in a study of 109 styrene exposed workers from the reinforced plastics industry (styrene exposures 14 to 39 ppm) and 54 controls no effects on the frequency of chromosomal aberrations or SCE (70 exposed, 31 control) were detected. The frequency of micronuclei, scored by the cytokinesis-block method among non-smokers also indicated no differences between 50 styrene exposed workers and 37 referents.

A field study was also conducted by Severi *et al* (1993) in which 52 workers exposed to styrene (up to 25 ppm) in the fibreglass-reinforced polyester resins manufacturing were monitored for a period of 4 wk. During this time blood samples were taken and lymphocytes analysed for chromosomal aberrations, SCE and micronuclei. No significant differences between the exposed and control population was found. For SCE a significant difference between smokers and non-smokers was however detected.

Perhaps the most comprehensive of all human cytogenetic investigations is the EEC Project on Biomonitoring of Human Population Exposure to Environmental Genotoxic Chemicals which integrates development, intercalibration of cytogenetic monitoring assays among various European laboratories and comparison of different cytogenetic endpoints. A survey performed in 32 workshops in the reinforced plastic industry with the concentration of styrene in the range from 5 - 182 ppm did not show any work related effects in the cytogenetic parameters (chromosome aberrations, sister chromatid exchanges or micronuclei) analysed in peripheral blood lymphocytes (Sorsa *et al*, 1991). Furthermore, the authors concluded that comparison of the laminate workers with other reinforced plastics workers showed no significant dose-dependent effects in the cytogenetic parameters.

Only 3 out of 14 studies have reported a significant increase in SCE in styrene workers. Of these the studies reported by Meretoja *et al* (1977) and Camurri *et al* (1983 and 1984) have already been discussed. The only other study reporting a relationship between styrene exposure and increased SCE frequency is the investigation by Yager *et al*, (1990) who reported elevated SCE at average styrene exposure below 40 - 50 ppm in a boat manufacturing facility. While the study may have been appropriate to determine potential benefits of the use of longitudinal sampling design, several deficiencies in the publication make it difficult to interpret the results. For example no data were presented in the paper to allow the reader to fully understand whether or not there was a statistically significant difference in SCE frequency between the groups. Blood samples were collected on three occasions but only the results of two sampling times were apparently scored. The most striking element is however the apparent lack of a control group. In addition to these problems it is unclear how the workers were selected, since none of them were exposed to concentrations of styrene above 55 ppm. Since it is impossible to make valid conclusions without comparison of SCE frequency in exposed workers to those in an unexposed population this publication, based on preliminary results, must be considered to contribute very little, if anything, to an understanding of the genotoxic potential of styrene.

In agreement with an examination of the chromosomal aberration investigations the better conducted SCE studies, including the largest numbers of individuals, have also proven to be negative.

The lack of aberrations and SCE is also in agreement with the data on micronuclei (Table 13) which show that the majority of the better studies provide little or no evidence for an association between styrene exposure and an increase in micronuclei. Of the positive studies Hogstedt *et al* (1983), Nordenson and Beckman (1987) and Brenner *et al* (1991) reported an increase in micronucleus frequency in workers with average exposures of only 13, 24 and 11 ppm respectively - in the study by Hogstedt *et al* (1983) the authors reported a remarkable increase in micronuclei almost 5 fold higher than control values. By contrast Maki-Paakkanen *et al* (1991) , Sorsa *et al* (1991), Norppa *et al* (1991) and Severi *et al* (1993) saw no increases in workers with much higher exposures i.e. 70 ppm, 43 ppm (maximum 162 ppm), 17 to 39 ppm and 25 ppm. These latter studies, included adequate numbers of individuals, and an improved methodology using cytochalasin-B (to prevent cytokinesis).

7.3.2 Summary of Human Cytogenetic Studies

For more than a decade, there have been conflicting reports of cytogenetic effects from styrene exposure (for a review, see Barale, 1991). As a general observation many of the studies describing either positive or negative effects have limitations because of the number of persons tested, time of sampling, tissue culture medium, classification of aberrations, and failure to account for possible confounding factors in comparing exposed and control groups. In an evaluation of cytogenetic effects of styrene Scott and Preston (1994) concluded that the positive findings reported in some papers are not compatible with the conclusion that styrene is responsible for the cytogenetic effects. The following reasons are given:

- the positive and negative effects do not appear to have any relationship with level of exposure i.e. lack of a dose response effect.
- induction of SCE and chromosomal aberrations seen in workers are opposite to that seen in experimental animals (see below).
- the types of chromosome changes are inconsistent with effects reported in cultured cells.
- reports of SCE induction in workers exposed to low concentrations of styrene are not compatible with effects seen in animals particularly in view of differences in biokinetics between the species.

They conclude that cytogenetic effects reported in some studies are probably attributable to the presence of clastogenic materials in the workplace as yet unidentified.

7.3.3 Cytogenetics - Rodent Studies

Problems encountered in cytogenetic studies of human populations such as mixed exposures and difficulties in accurately assessing exposure levels can be overcome in well conducted animal investigations. To date there have been a number of cytogenetic studies in experimental rodents using both styrene (Tables 14 and 15) or SO (Table 16). An examination of Table 14, which summarises the data on chromosomal aberrations in bone marrow from rodents treated with styrene, shows that all of the studies except one gave negative results. The one study which produced a positive effect was conducted by Meretoja *et al* (1978a) who analysed rat bone marrow cells after inhalation of styrene. Exposures were to 300 ppm for 2-11 wk (6 h/d, 5 d/wk), with animals being sampled weekly, with the majority of animals in the 7 to 11 wk groups. No increase in aberration frequencies were observed in the styrene-treated group compared to the control during the first 8 wk of exposure. At longer exposure times the authors report an increase in aberrations. The results are quite difficult to understand especially why all data were negative up to eight wk of exposure and then positive there after. Also the authors state that most of the aberrations are "chromosome-type breaks" whereas the vast majority of chemical agents induce chromatid-type aberrations. In addition bone marrow cells are dividing, and deletions either a) induce cell killing and/or b) are lost from the daughter cells at mitosis. Since any observed deletions must have been produced in the cell cycle immediately prior to the metaphase analysed it is difficult to explain the positive effects occurring only after eight wk exposure. Such problems make it very difficult, if not impossible, to explain the data thereby invalidating any conclusion that styrene has been shown to be clastogenic *in vivo*.

Norppa *et al* (1980b) also conducted a study on the effects of styrene inhalation exposures on the induction of chromosome aberrations in Chinese hamster bone marrow cells. Exposures were 300 ppm styrene for 4 d or 3 wk (6 h/d, 5 d/wk) and bone marrow samples were taken immediately after the termination of exposure. There was no increase in chromosome aberrations for either treatment. In addition, there was no increase in aberrations when styrene exposures were given in combination with 15% ethanol in the drinking water.

Perhaps the most convincing study that styrene does not induce chromosomal aberrations in experimental rodents can be found in the studies by Kligerman *et al* (1992 and 1993) and Preston and Abernathy (1993). In the investigations conducted by Kligerman *et al* (1992 and 1993) B6C3F1 mice and F-344 rats were exposed by inhalation for 6 h/d for 14 d to nominal concentrations of either 0, 125, 250, or 500 ppm styrene. One day after the final exposure mouse lung, peripheral blood and spleen cells were analysed for increase in micronuclei (MN) induction, chromosomal breakage and SCE induction. The authors reported a small but statistically significant increase in SCE frequencies of the mouse lung cells, splenic and peripheral blood lymphocytes as well as in the peripheral blood lymphocytes of the rat. No increase was seen in chromosomal aberrations or micronuclei in any of the cells collected from the mouse or rat, indicating that styrene, even at very high exposure levels, does not appear to produce an increase in chromosomal aberrations or micronuclei. In the study by Preston and Abernathy (1993) Fischer 344 rats were exposed to styrene by inhalation for 6 h/d, 5 d /wk for 4 wk at nominal concentrations of 0, 150, 500 or 1,000 ppm. Ethylene oxide

(150 ppm) was included as the positive control. Reciprocal blood cell cultures were established 1,2,3 and 4 wk after the start of exposure. 4 wk after termination the cells were examined for chromosomal aberrations and SCE. No treatment related effects were observed at any time point for any treatment level. Thus in very extensive studies, examining both time and treatment variables, styrene did not produce chromosomal aberrations even at very high exposure levels.

Other cytogenetic studies measuring SCE or micronuclei in rodents exposed to styrene are shown in Table 15. Conner *et al* (1979, 1980) reported an increase in SCE in regenerating liver and bone marrow of mice exposed to concentrations of 565 to 922 ppm, 6 h/d for 4 d. At lower exposures i.e. 100 to 387 ppm, 6 h/d for 4 d no effects on SCE in bone marrow or regenerating liver were seen. Using the inhalation route Kligerman *et al* (1992 and 1993) reported small but statistically significant concentration increases in SCE in both mice and rats exposed to 125, 250 or 500 ppm styrene 6 h/d, 5 d/wk for 2 wk. It is interesting to note that both rat and mouse showed similar modest effects in SCE induction. The rat showed a slightly steeper dose response slope even though, due to interspecies differences in biokinetics, the mouse will have substantially higher blood and tissue concentrations of styrene oxide. In their very extensive study in the rat Preston and Abernathy (1993) failed to find any evidence for styrene exposure producing SCE. It has been suggested that the SCE seen by Kligerman (1992 and 1993) may have been influenced by the high concentrations of bromodeoxyuridine used for staining the cells; this is to be verified.

A variety of studies have been undertaken with the putative clastogen styrene oxide (see Table 16). Of these studies the majority have failed to find any evidence for a clastogenic effect even though subsequent biokinetic investigations suggest that the exposure regimens used in the studies will have produced high internal doses of SO.

Table 14: Chromosomal Aberrations in Bone Marrow from Rodents Treated with Styrene

Species	Route of Exposure	Dose Range	Result	Reference
Mouse	Oral	500 to 1,000mg/kg body wt (single dose)	N	Loprieno *et al* (1978)
Rat	Inhalation	300 ppm (6h/d, 5 d/wk for 9 wk	N	Merejota *et al* (1978b)
Rat	Inhalation	300 ppm (6h/d, 5 d/wk for 11 wk	P	Merejota *et al* (1978a)
Chinese Hamster	Inhalation	300 ppm (6h/d, 5 d/wk for 4 to 21 d	N	Norppa *et al* (1980)
Mouse	Oral	500mg/kg bw, 4 d treatment	N	Sbrana *et al* (1983)
Mouse	Oral	200mg/kg bw, 70 d treatment	N	Sbrana *et al* (1983)
Rat	Inhalation	600 or 1,000 ppm (6h/d, 5 d/wk for 52 wk	N	Sinha *et al* (1983)
Mouse	Inhalation	125, 250 or 500 ppm (6h/d, 5 d/wk for 14 d	N	Kligerman *et al* (1992)
Rat	Inhalation	125, 250 or 500 ppm (6h/d, 5 d/wk for 14 d	N	Kligerman *et al* (1993)
Rat	Inhalation	0, 150, 500 or 1,000 ppm, 6 h/d, 5 d / wk for 4 wk	N	Preston and Abernathy (1993)

N = negative result
P = positive result

Table 15: Sister Chromatid Exchanges (SCEs) and Micronuclei (MN) In Peripheral Blood lymphocytes (PBL), Bone Marrow (BM) or Regenerating Liver (RL) Obtained from Rodents Treated with Styrene

Species	Route of Exposure	Dose Range	Assay	Tissue	Result	Reference
Mouse	Inhalation	565 to 922 ppm (6 h/d, 4 d)	SCE	RL	P	Conner *et al* (1979, 1980)
Mouse	Inhalation	100 to 387 ppm (6 h/d, 4 d)	SCE	RL	N	Conner *et al* (1979, 1980)
Mouse	I.P. injection	50 to 1,000 mg/kg	SCE	BM	N	Conner *et al* (1979, 1980)
Chinese Hamster	I.P. injection	1.0 g/kg	MN	BM	N	Penttila *et al* (1980)
Mouse	Inhalation	125, 250 or 500 ppm (6 h/d, 5 d/wk for 14 d)	SCE	*PBL	**P	Kligerman *et al* (1992)
Rat	Inhalation	125, 250 or 500 ppm (6 h/d, 5 d/wk for 14 d)	SCE	PBL	**P	Kligerman *et al* (1992)
Rat	Inhalation	0, 150, 500 or 1,000 ppm, 6 h/d, 5 d/wk for 4 wk	SCE	PBL	N	Preston and Abernathy (1993)

N = negative result
P = positive result

* positive effect also seen in spleen and lung cells
** small but statistically significant concentration related increase in SCE

Table 16: Micronuclei (MN) In Bone Marrow (BM), Alveolar Macrophages (AM) or Regenerating Liver (RL) from the Mouse Treated with Styrene Oxide

Species	Route of Exposure	Dose Range	Assay	Tissue	Result	Reference
Mouse	I.P. injection	250mg/kg	CA MN	BM BM	N N	Farby *et al* (1978)
Mouse	Oral	50mg/kg	CA	BM	N	Loprieno *et al* (1978)
Mouse	Oral	500 or 1,000mg/kg	CA	BM	P	Loprieno *et al* (1978)
Mouse	Inhalation	25 to 100 ppm (5 h exposure)	CA SCE	BM BM	N N	Norppa *et al* (1979)
Mouse	Inhalation	50 ppm (6h/d, 4 d)	SCE SCE SCE	RL BM AM	N N N	Conner *et al* (1982)

N = negative result
P = positive result

7.3.4 Summary of Rodent Assays

From a consideration of all the rodent data, it can be concluded that styrene, or indeed SO, is not effective at inducing chromosome aberrations in rodent cells. Despite some inadequacy in the protocols used for *in vivo* cytogenetic assays, styrene does not induce chromosome aberrations in mouse bone marrow cells, peripheral blood, spleen and lung cells. High exposures to styrene do not cause an increase in micronuclei. While there is some limited evidence that at exceptionally high doses styrene may produce a small increase in SCE in mouse bone marrow cells, spleen and peripheral blood cells it should be remembered that 1) the majority of human studies do not show an increase in SCE frequencies and 2) at inhalation exposures to styrene of 500 ppm the SO detoxification mechanism in the mouse becomes overwhelmed resulting in extremely high systemic exposures of the oxide (see Section 4 - Toxicokinetics). The toxicokinetic data show that the blood SO levels in the mouse exposed to 500 ppm styrene are about 3,000 fold higher than levels in man at a workplace exposure of 20 ppm styrene (ECETOC Special Report No. 3, 1992).

7.3.5 Cytogenetics - *in vitro* Data

There is limited data available on the clastogenic effects of styrene in Chinese hamster cells (3 publications) and five original reports on treatment of human lymphocytes *in vitro*. The same data however appear in different publications. A review of these studies has already been published (Preston, 1990). The data are summarised in Table 17.

Matsuoka *et al* (1979) and de Raat (1978) reported the absence of chromosomal aberrations and SCE, respectively, in chinese hamster cells treated with styrene in the absence of S9 metabolic activation system - dose range 10 to 250 µg/ml. Both authors reported clastogenic responses when the cells were incubated with styrene in the presence of S9,- dose range 250 to 1,000 µg/ml - De Raat added cyclohexene oxide as an inhibitor of epoxide hydratase, the enzyme that would normally metabolise styrene oxide. Because Matsuoka *et al* (1979) included gaps as clastogenic effect (gaps are not considered true aberrations - Preston *et al*, 1987) their data cannot be properly analysed to determine the potential clastogenicity of styrene or its metabolites Preston (1990).

De Raat (1978) also examined the ability of styrene oxide to produce SCE in Chinese hamster cells (concentration range approximately 25 to 100µg/ml) and Turchi *et al* (1981) studied anaphase bridges and micronulclei (end points as observed by Preston (1990) to be very poor for assessing clastogenic effects) in cells treated with styrene oxide at a single concentration of approximately 80µg/ml. De Raat reported that styrene oxide in the absence of S9 was an effective inducer of SCE. In the presence of S9 no effects were seen, presumably due to the rapid detoxification of the oxide. While the study can be criticised for a variety of reasons (Preston, 1990) Turchi *et al*, reported that treatment resulted in an increased frequency of both anaphase bridges and micronulclei.

Table 17 Summary of *in vitro* Cytogenetic Assays with Styrene

Cell type	Endpoint	Metabolic activation	Dose range (µg/ml)	Result	Reference
H L	CA	+	3,000	P	Linnainmaa *et al* (1978)
H L	SCE	+	34 to 416	P	Norppa *et al* (1980)
H L	SCE	+	52 to 416	P	Norppa and Vainio (1983)
H L	SCE	+	approx 200	P	Norppa *et al* (1983)
H L	SCE	-	102 to 2,040	N	Norppa and Tursi (1984)
H L	SCE	+	102 to 2,040	N	Norppa and Tursi (1984)
H L	SCE	RBC	102 to 2,040	P	Norppa and Tursi (1984)
H L	SCE	RBC	50 to 400	P	Norppa *et al* (1985)
H L	CA	+	6 to 60	N	Pohlova *et al* 1985
H L	CA	-	100 to 625	WP	Jantunen *et al* (1986)
H L	CA	-	100 to 625	WP	Jantunen *et al* (1986)
C H	CA	+	250	P	Matsuoka *et al* (1979)
C H	CA	-	250	N	Matsuoka *et al* 1979
C H	SCE	+	500 to 1,000	P	de Raat (1978)
C H	SCE	+/-	10 to 100	N	de Raat (1978)

\+ : with metabolic activation
\- : without metabolic activation
N : negative result
P : positive result
WP : very weak positive
CA : chromosomal aberration
SCE : sister chromatid exchange
RBC : red blood cells
HL : human lymphocyte
CH : Chinese hamster

7.3.6 Summary and Evaluation of Cytogenetic Data

While it is agreed generally that *in vitro* tests can provide suspicions of possible adverse effects their predictive value is much lower than that of animal studies (Henschler, 1987). This is supported by an analysis of the relationship between mutagenic and carcinogenic potency (Krewski *et al*, 1992, 1993) which showed that the overall scatter was sufficiently great to preclude the use of the Ames test as a predictor of carcinogenic potency. Thus for human hazard assessment conclusive evidence that a chemical poses a heritable hazard can only come from *in vivo* studies.

To date the largest data base on the putative genotoxic potential of styrene are results of studies on workers, mainly employed in the reinforced plastics industry, investigating whether or not styrene exposure (usually at relatively high concentrations) is associated with evidence of chromosomal damage (Barale, 1991). All of these studies have used peripheral lymphocytes as the most convenient source of cells for analysis. Approximately one third of the studies have reported significant increases in chromosomal damage (including aberrations and/or SCE) compared with controls while the remaining two thirds have reported no effects associated with exposure to styrene. An examination of the positive results shows no quantitative relationship between exposure and chromosome damage for example chromosomal aberrations have been reported at exposures as low as about 10 ppm Camurri *et al*, 1983, which is in striking contrast to negative responses reported in individuals with exposures up to 180 ppm (Sorsa *et al*, 1991). Of the studies undertaken those showing negative responses are technically the most advanced in terms of the number of exposed individuals and matched controls as well as methodological protocol. After a review of the available data (Scott, 1993) concluded that the human findings were not consistent with the interpretation that styrene is responsible for the observed positive results in the workers and that the positive responses may simply be a result of either chance (as a result of the low numbers of samples examined), unacceptable methodology resulting in false positives or the presence of clastogenic chemicals in the workplace. Animal studies with styrene or styrene oxide have generally failed to show evidence for either chemical producing chromosomal damage although there is a suggestion that styrene may increase the incidence of SCE in experimental rodents. This latter observation is quite interesting since the vast majority of studies examining the incidence of SCE in humans have proven negative (Table 12).

It is very difficult to reach a firm conclusion about the putative clastogenic potential of styrene and it is equally difficult to know what type of studies will help resolve the issue. As described above well conducted, state of the art animal studies are in the main negative (Preston and Abernathy, 1993, Kligerman *et al* 1993) and it is questionable if other studies could add to our knowledge. Conducting further human studies may also not be very helpful since it has been proposed that positive effects occur only with excessive exposures, i.e. positive results are in the main from investigations conducted 10 or more years ago when exposures, to styrene and other chemical agents, in the reinforced plastics industry were higher than those found currently. Thus while there may be the odd study (e.g. Tomanin *et al*, 1992) reporting chromosomal damage at current occupational guidelines it is agreed, generally, that today, in Western Europe, chemical exposures in the reinforced plastics industry are at levels below which chromosomal damage will occur. With a shortage of suitable populations in Western

Europe reports are beginning to emerge from developing countries such as Egypt (Anwar and Shamy, 1993) while blood samples taken from individuals in former soviet block countries are finding their way westwards for examination. There must however be a large question mark over the value of such investigations since pragmatism dictates that qualitative and quantitative information on exposures to styrene and/or other chemicals may be less than perfect. Also there must be some concern over the social, health and environmental conditions which such populations find themselves in, and the related confounding factors. In the absence of any forthcoming decisive information it may only be possible to conclude that if exposure to styrene ever did produce chromosomal aberrations the levels required to produce such effects are greater than those encountered in today's work place.

7.4 GENOTOXICITY - MUTAGENICITY

7.4.1 Microbial Systems

Styrene is not mutagenic to any of *Salmonella typhimurium* strains in the absence of a mammalian metabolic activating system. In the presence of an activating system, no positive results have been reported in the *Salmonella* strains TA98, TA1537 and TA1538, which are capable of detecting frame-shift mutations.

A few studies have reported a positive response (Table 18) in the presence of an S9 metabolising system and when epoxide hydratase activity was inhibited. There are a variety of reasons for the conflicting results as described below.

Table 18: Summary of Mutagenicity Assays with Styrene in *Salmonella typhimurium*

Metabolic activation	Dose range (mg/plate)	Result	Reference
-	7.0	N	Milvi and Garrow (1976)
+	0.0001 to10.0	P	Vainio *et al* (1976)
-	0.0001 to 10.0	N	Vainio *et al* (1976)
+/-	0.0001 to 10.0	N	Stoltz and Withey (1977)
+	0.0001 to 0.1	P	De Meester *et al* (1977)
-	0.0001 to 0.1	N	De Meester *et al* (1977)
+	0.0001 to 1.0	N	Loprieno *et al* (1978)
+	312 to 624	P	Watabe *et al* (1978a)
+	0.0001 to 0.1	N	Busk (1979)
+	1.04	P	Poncelet *et al* (1980)
+	1.04	N	Poncelet *et al* (1980)
+	gaseous	N	Poncelet *et al* (1980)
+	gaseous	E	De Meester *et al* (1981)
+	up to 0.7	N	De Flora (1981)
+	up to 0.7	N	De Flora (1981)
+/-	0.0003 to 0.33	N	Dunkel *et al* (1985)
+	0.01 to 0.1	N	Brams *et al* (1987)

+ : with metabolic activation
- : without metabolic activation
+/- : with or without metabolic activation
N : negative result
P : positive result
E : equivocal

Vainio *et al* (1976) reported a low level positive response in a plate test with TA100 and TA1535. An increase in the number of revertants occurred over a very narrow dose range of 2-11 µmole/plate. The authors suggested that the high toxicity of styrene to the tester cells made interpretation of the results difficult. However, the most important cause of the conflicting results seems to be due to the presence of *diethyl maleate*, which was added to deplete cytoplasmic glutathione, and 3,3,3-trichloropropene oxide, to inhibit epoxide hydratase. These inhibitors were presumed to enhance the mutagenicity of styrene by interfering with the inactivation of the metabolite styrene oxide.

Conflicting results have been reported when styrene was tested with *Salmonella* strains TA100, TA1530 and TA1535 in the presence of an activating system (De Meester *et al*, 1977, 1981). De Meester *et al*, (1981), suggested that the poor solubility of styrene and the fact that styrene has relatively high volatility may also be responsible for the discrepancies in the literature regarding styrene mutagenicity. These investigators conducted a vapour phase experiment on styrene. The authors reported that styrene (24% v/v styrene/air) showed a weak mutagenic activity in the presence of Aroclor 1254-induced rat liver S9. A technique to convert liquid styrene to a vapour phase was not presented in the paper and the method of monitoring the concentration of styrene in the desiccator was not described. It is inexplicable why the authors did not observe a toxicity at the reported top dose-level of 40% v/v which in fact exceeds the maximum vapour pressure for styrene. Further, the results were presented only in the form of graphs; no experimental and positive control values were included. Considering that the modified protocols can only be successful if provision is made for repeat tests in cases where suspicions are raised about the validity of test response, most of this study value is lost.

Although Watabe *et al* (1978a and b) reported styrene to be mutagenic in *Salmonella* strain TA100, examination of the reported data revealed that a mutagenic response was not induced, and that the apparent positive response was the result of an artefact arising from the protocol used (Dunkel *et al*, 1985).

Yoshikawa *et al* (1980) and El-Tantawy and Hammock (1980) have observed the rapid metabolism of styrene to non-mutagenic compounds by epoxide hydratase and glutathione-S-transferase in the S9 fraction of rat liver. This observation could provide further explanation for the lack of mutagenicity of styrene in the *Salmonella typhimurium* mutagenicity assay.

Perhaps the most intensive and influential investigation of the mutagenic potential of styrene is that reported by Dunkel *et al* (1985). In this inter-laboratory investigation styrene was examined for mutagenic potential in four independent laboratories using *Salmonella typhimurium* and *E. coli* WP2 UVR A assay systems. Identical protocols were used which included six different metabolic activation systems derived from three species (induced and non-induced). All the four laboratories failed to find any evidence of mutagenic effects and unanimously reported a clear-cut negative response.

Pooling the data from other individual studies on mutagenicity of styrene leads to the conclusion that styrene does not produce microbial mutagenicity with and without a metabolic activation system (Busk, 1979; de Flora, 1981; Stoltz and Witney, 1977; Loprieno *et al*, 1978).

The variability in response in the presence of S9 (not in its absence) serves to highlight the difficulty of utilising exogenous activation as a "model" for *in vivo* effects.

7.4.2 Mutagenicity - Eukaryotic Systems

Styrene has been tested *in vitro* for mutagenicity in yeast and in mammalian cells in culture. Loprieno *et al* (1976; 1978) observed no increases in forward mutations in *Schizosaccharomyces pombe* or in gene conversions in *Saccharomyces cerevisiae* exposed to styrene with and without metabolic activation (purified microsomes from mouse liver). When metabolic activation was provided through mice given styrene by gavage at a dose of 1,000 mg/kg in a host-mediated assay, there was still no increase in forward mutations in *S. pombe*; however, there was an increase in gene conversion at the ade locus in *S. cerevisiae*.

Styrene showed positive results in *Drosophila melanogaster* at exposures of 200 ppm by feeding (Donner *et al*, 1979). Negative results were observed for *D. malanogaster* 100 ppm for 2.5 h inhalation (McGregor, 1981).

Styrene induced point mutations in chinese hamster V79 cells, but only with the liver perfusion system (Beije and Jensson, 1982). However, Loprieno *et al* (1976) did not observe mutagenic activity in treated Chinese hamster V79 cells with either 0.85 or 17 mM styrene for 1 hour.

7.4.3 Summary and Evaluation of Mutagenicity Data

Styrene does not appear to be a direct acting mutagen and/or germ-cell mutagen. Even in the presence of activation, a positive response seems to occur only in a rather narrow range with Salmonella strains TA100 and TA1535.

7.5 DNA BINDING STUDIES

The somatic cell mutation theory of carcinogenesis suggests that cancer is caused by genetic damage, and that mutations resulting from covalent interactions of chemicals with DNA may be an important initial stage in chemical carcinogenesis. DNA binding studies and the mechanism of DNA damage may therefore be important considerations in identifying potential carcinogens.

Byfalt-Nordqvist (1985) investigated the extent of covalent binding to guanine N-7 in DNA isolated from mice treated i.p. with radiolabelled styrene or SO. The authors reported that liver DNA binding was 17±5 or 8±2 nmole guanine N-7 adduct per kg bw in mice given 1 mmole/kg of styrene or SO, respectively. This observation seems inconsistent with styrene and SO toxicokinetics since internal SO dosimetry is far higher in mice given SO than in those given an equivalent dose of styrene (see Toxicokinetics section). The reported DNA binding in mouse liver could not be reproduced in more recent studies by Cantoreggi and Lutz (1992; 1993).

In an initial series of studies, Cantoreggi and Lutz (1992) administered radiolabelled SO to rats by oral gavage at dose levels of 1.65 or 240 mg/kg. Radiolabelled SO was also given to mice by i.p. injection using the same dosing regimen as reported by Byfalt-Nordqvist. After 4 or 24 h, DNA from the forestomach, glandular stomach and liver of rats was isolated and purified for determination of radioactivity. At the 4-h time point, radioactivity in the DNA was below the limit of detection in the forestomach and liver. Expressed in units of the Covalent Binding Index (CBI = µmole adduct per mole DNA nucleotide/mmole chemical administered per kg bw), the DNA-binding potency (i.e., CBI) in the forestomach was below 2.6 and 2.0, respectively. DNA samples from tissues collected 24 h post-treatment and the glandular stomach collected 4 h post-treatment contained small amounts of radioactivity. Enzymatic degradation of the DNA and separation of the normal nucleotides showed, however, that this radioactivity represented biosynthetic incorporation of radiolabel into newly synthesized DNA rather than covalent binding. The limit of detection for DNA adducts in the glandular stomach was at 1.0. The hepatic DNA from mice 2 h after injection of SO showed no radioactivity at a detection limit of 0.6, and thus was at least 40-fold lower than indicated by Byfalt-Nordqvist *et al* (1985). The authors concluded that the chemical reactivity of SO appears to be too low to result in a detectable production of DNA adducts in an *in vivo* situation. Furthermore, the authors concluded that a purely genotoxic mechanism of tumour action by SO is unlikely, and that the observed SO tumorigenic response in the rodent forestomach is probably the result of strong tumour promotion by regenerative hyperplasia.

Subsequent studies were conducted by Cantoreggi and Lutz (1993) to evaluate DNA binding following *in vivo* exposure to styrene. Male and female rats and mice were exposed in a closed chamber system to radiolabelled styrene at peak concentrations of 200 and 400 ppm. No significant differences were seen between mice and rats or between sexes. The metabolised doses were calculated to be between 20 and 39 mg/kg in the rats, and between 70 and 110 mg/kg in the mice. Liver DNA (from both rats and mice) and lung DNA (from rats only) was purified and analysed for nucleotide adducts. The mouse liver DNA showed a minute but significant amount of radioactivity which eluted at retention times seen for adducts

prepared from DNA and SO *in vitro*; the DNA-binding potency was however extremely low, averaging at 0.1 CBI units. Analysis of DNA samples from the rat showed no adduct formation in the liver; some radioactivity was detected in the lung tissue, but the binding index was extremely low (0.07 CBI units). The DNA binding in liver of mice exposed to styrene was 200-500 times lower than was reported by Byfalt-Nordqvist *et al* (1985). The differences in DNA binding of SO and styrene in comparison to Byfalt-Nordqvist *et al* (1985) was thought to be due to insufficient purification of the DNA the Byfalt-Nordqvist group to ensure that their DNA samples were completely free of non-covalently bound radiolabelled styrene metabolites. The results of these studies show that styrene metabolism in the rat or the mouse does not result in the formation of any potent DNA-binding intermediates. The authors concluded that the low level of DNA adduct formation by styrene is highly unlikely to be responsible for a significant increase in tumour incidence in a standard animal bioassay. Any theoretical risk to humans would likewise be extremely low.

Walles and Orsen (1983) reported single strand breaks in DNA isolated from mice given styrene or SO by i.p. injection. The authors reported that strand breaks increased as a linear function of dose and that a 2-fold higher dose of styrene was required to produce equivalent effects from SO. A linear correlation between strand breaks and the applied dose wasreported over an SO dosage range from 1 to 7 mmole/kg (i.e. 120 to 840 mg/kg). This observation is however inconsistent with biokinetic data which shows that, because the clearance mechanism becomes overwhelmed, internal dose of SO increases approximately 50-fold even though the applied dose only increased 5-fold i.e. 100 to 500 mg/kg (ECETOC, 1992). Thus one would expect a non-linear increase in damage with increasing dose levels. The results reported by Walles and Orsen (1983) are also inconsistent with those of Kligerman *et al* (1993) who, using a single cell assay under alkaline conditions, failed to find DNA strand breaks in lymphocytes from rats exposed by inhalation to styrene for 6 h/d for 14 consecutive d at concentrations of 0, 125, 250 or 500 ppm.

Mäki-Paakkanen *et al* (1991), Brenner *et al* (1991) and Walles *et al* (1993) reported the examination of DNA single strand breaks (SSB) in leucocytes from workers exposed occupationally to styrene. In the study by Mäki-Paakkanen *et al* (1991) SSB were determined in 9 exposed workers (6 male smokers, 1 male non-smoker and 2 female smokers) and 8 controls (3 male and female smokers, and 2 male non-smokers). The authors reported "an increase in SSB" although no further details were given. Brenner *et al* (1991) examined SSB levels in 14 exposed workers and 8 controls. No significant association was seen between SSB and styrene exposure. Smoking did appear to have a significant effect on the frequency of breaks. In the study by Walles *et al* (1993) SSB in DNA were monitored in leucocytes from 17 men occupationally exposed to styrene at an estimated exposure concentration of about 20 ppm. Of the 17 men 10 were smokers at the time of investigation, 6 men were taking snuff and 6 were taking pharmaceutical medication. No non-exposed controls or non-smokers were apparently included in the investigation. The authors reported a good correlation between SSB and styrene exposure at 18 ppm with levels of SSB apparently doubling after 8 h exposure, i.e. SSB higher in leucocytes collected after the work shift as compared to samples taken just prior to the work shift. The authors also noted that smoking may increase SSB. The highest level of SSB was found in a man who had taken paracetamol medication.

7.6 ANIMAL - CARCINOGENICITY STUDIES

Six chronic toxicity/carcinogenicity studies on styrene have been conducted in rats, while three such studies have been conducted in mice. In addition, a rat and a mouse study have been conducted on a mixture of 70% styrene and 30% β-nitrostyrene. Each of the studies will be reviewed and an overall assessment provided.

Because styrene is metabolized largely by conversion to styrene-7,8-epoxide (commonly called styrene oxide), which is further metabolized, carcinogenic effects from styrene oxide may be relevant to the assessment of the carcinogenic effects of styrene. Three oral and two dermal carcinogenicity studies have been conducted on styrene oxide.

7.6.1 Styrene Oral Administration to Rats

Pregnant BDIV rats were given a single gavage dose of 1350 mg/kg styrene in olive oil on day 17 of gestation; their progeny (73 males and 71 females) were given 500 mg/kg of styrene once each week for life. A control group (36 males and 39 females) received only olive oil.

As concluded by the authors, there was no evidence of carcinogenicity in this study (Ponomarkov and Tomatis, 1978).

In the National Cancer Institute study (NCI, 1979), Fischer 344 rats received styrene in corn oil at doses of 1,000 or 2,000 mg/kg/d 5 d/wk for 78 wk, with observation for an additional 27 wk. Because the high incidence of mortality at 2,000 mg/kg/d precluded evaluation of the risk for late-developing tumours, an additional group was administered 500 mg/kg/d 5 d/wk for 103 wk. This study is of major importance because it provided an evaluation of carcinogenic response at maximum tolerated doses. As concluded by the authors, there was no evidence of tumours at 500 or 1,000 mg/kg/d.

Maltoni's laboratory administered styrene in olive oil by gavage to Sprague-Dawley rats four to five d/wk for 52 wk at doses of 50 or 250 mg/kg/d (Conti *et al*, 1988); the animals were observed for tumour development for life. As concluded by the authors, there was no evidence of carcinogenicity in this study.

Styrene was administered in the drinking water of Sprague-Dawley rats for two years at concentrations of 125 and 250 ppm (a saturated solution of styrene in water at 25°C contains 320 ppm styrene). During the experiment, some males and females were selected for breeding to provide two additional generations to examine styrene's potential to produce reproductive effects. Those used for breeding were not sacrificed, but continued to receive styrene in their drinking water for the remainder of the two years. Calculated styrene intake was 7.7 and 14 mg/kg/d for males and 12 and 21 mg/kg/d for females (Beliles *et al*, 1985). As concluded by the authors, there was no evidence of carcinogenicity in this study.

The NCI also conducted a study in Fischer 344 rats using a mixture of 70% styrene and 30% β-nitrostyrene. The styrene administered amounted to 350 or 700 mg/kg/d three d /wk for 79 wk in males and 175 or 350 mg/kg/d in females: the rats were observed for tumor formation

for an additional 29 wk (NCI, 1978). As concluded by the authors, there was no evidence of carcinogenicity in this study.

7.6.2 Styrene Oral Administration to Mice

Pregnant C57B1 mice were given a single gavage dose of 300 mg/kg styrene in olive oil on day 17 of gestation; their progeny (27 males and 27 females) were given 300 mg/kg of styrene once each week for life. One control group (13 males and 12 females) received only olive oil, while a group of 51 males and 49 females served as untreated controls. Because there were no significant differences in tumor incidence, there was no evidence of carcinogenicity in this study (Ponomarkov and Tomatis, 1978).

Pregnant O20 mice were given a single gavage dose of 1350 mg/kg styrene in olive oil on day 17 of gestation, their progeny (45 males and 39 females) were given 1350 mg/kg of styrene once each week for 16 wk and observed for life. One control group (20 males and 22 females) received only olive oil, while a group of 54 males and 47 females served as untreated controls. Treatment was terminated at 16 wk because the styrene-treated mice had a much higher incidence of mortality than either control group. The authors reported that males had a statistically significant increase in lung tumours compared to the olive oil control, but not the untreated control, while the females had increased lung tumours compared to the untreated control, but not the olive oil control (Ponomarkov and Tomatis, 1978).

The authors recognized the shortcomings of this study in their conclusions:

"The increased incidence and early appearance of lung tumours could possibly indicate a carcinogenic effect for styrene in O20 mice. This experiment, however, has severe limitations, since the dose used was very high, causing severe toxic effects and early mortality. Additional studies are needed before a final evaluation of the carcinogenicity of styrene in rodents can be made."

Interpretation of this study is complicated by the small numbers of animals in the study, inconsistent differences between which control group was different from the styrene-treated mice, the absence of data on litter effects, the absence of historical background lung tumor incidence, and by the fact that the Maximum-Tolerated-Dose was clearly exceeded.

In the National Cancer Institute study (NCI, 1979), B6C3F1 mice received styrene in corn oil at doses of 150 or 300 mg/kg/d 5 d/wk for 78 wk, with observation for an additional 13 wk. Treatment was stopped at 78 wk because of the high incidence of mortality at 300 mg/kg/d. The incidence of combined alveolar/bronchiolar carcinomas and adenomas was 0% in control males, 14% in low dose males and 21% in high dose males. Although these differences in tumor rates were statistically significant compared to the control within this study, the historical incidence in controls averaged 12% at this laboratory, which was not statistically different from the treated animals. The authors of this study concluded "that under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in Fischer 344 rats or B6C3F1 mice of either sex."

The NCI also conducted a study in B6C3F1 mice using a mixture of 70% styrene and 30% β-nitrostyrene. The styrene administered amounted to 407 mg/kg/d in males and 203 mg/kg/d in females three d /wk for 79 wk. The mice were observed for tumor formation for an additional 14 wk (NCI, 1978). As concluded by the authors, there was no evidence of carcinogenicity in this study.

7.6.3 Styrene Inhalation Studies with Rats

Maltoni and his associates conducted inhalation studies on styrene in the mid-1970s (one was simultaneous with the oral study listed previously); two reports appeared in the literature. Both reports indicate that Sprague-Dawley rats were exposed to 25, 50, 100, 200, or 300 ppm styrene 4 h/d, 5 d/wk for 52 wk. No increase in brain tumours from styrene exposure was reported based on groups of 40 males and 40 females (Maltoni *et al*, 1982). Six years later, data based on groups of 30 males and 30 females were reported that showed an increase in total and malignant mammary tumours in all groups treated with styrene (Conti *et al*, 1988).

The incidences were:

Exposure level (ppm)	0	25	50	100	200	300
Malignant mammary tumours (%)	10	20	13	30	40	30
Total mammary tumours (%)	57	80	70	71	80	83

The report does not indicate if the concurrent control group was placed in inhalation chambers while the styrene exposures were in progress; this is an important factor in conducting chronic inhalation studies. Also because of the high spontaneous incidence of mammary tumours in the strain of animals used historical values are considered important to allow a more definitive interpretation of the results. In fact, in 1978 Maltoni (a co-author with Conti) had pointed out that there was a high incident of mammary tumours in the rat colony at the institute where Conti conducted the study.

Another chronic inhalation study was reported in 1978; Sprague-Dawley rats were exposed to styrene at 600 or 1,000 ppm 6 h/d 5 d/wk; males were exposed for 18 months and observed for 6 more, while females were exposed for 21 months and observed for 3 additional months. Initially, the high dose was 1200 ppm; however, the concentration was reduced to 1,000 ppm after 2 wk because of early mortality in the males. An outbreak of murine pneumonia caused excessive mortality, particularly in the control and high-dose males. In females, the incidence of mammary gland adenocarcinomas was 1% in controls, 8% at 600 ppm, and 0% at 1,000 ppm. The incidence in the low dose group was statistically different from that of the control, although within the historical control range (0 to 9%, average 6%) of the laboratory. Also in females, there was a non-dose-related increase in the incidence of combined leukaemia/lymphosarcoma (1.2%, 7.1%, and 7.1% for 0, 600, 1,000 ppm, respectively). The incidence in treated animals was not statistically significantly different from

the combined controls. However, if the two dose groups were combined, the incidence was significantly different form historical controls. In males, the incidences were 1.2%, 5.9%, and 1.2%, respectively (Jersey *et al*, 1978). Since 1986, the National Toxicology Program has maintained that it is inappropriate to combine leukaemias and lymphomas. If considered separately, the incidences in the treated rats of leukaemia and lymphoma is not different than in the concurrent or historical control groups. This study provides no clear evidence for an oncogenic response resulting from inhalation exposures to styrene, but due to its limitations, it is inadequate for use in carcinogenic classification.

7.6.4 Exposure to Styrene by Other Routes

Maltoni's laboratory exposed rats to styrene by subcutaneous or intraperitoneal injections, as well as conducting the oral and inhalation studies previously discussed. One group of 40 male and 40 female rats received 4 intraperitoneal injections of 50 mg of styrene two months apart. Another group of 40 males and 40 females received one 50 mg subcutaneous injection of styrene. The rats in both groups were observed for tumor formation until death. Neither group had increased tumor incidences compared to controls.

7.6.5 Oral Studies of Styrene Oxide

Ponomarkov *et al* (1984) administered 200 mg/kg of SO in olive oil to pregnant BDIV rats on day 17 of gestation. After weaning, the progeny (43 males and 62 females) received weekly oral doses of 100 to 150 mg/kg SO in olive oil for 96 wk and were observed for a subsequent 24 wk. The incidence of forestomach tumours was increased in males and females.

Maltoni reported similar findings in male and female Sprague-Dawley rats exposed to 50 or 250 mg/kg/d of styrene oxide administered by gavage in olive oil 4-5 d /wk for 52 wk (Conti *et al*, 1988). He also reported hyperplastic lesions, papilloma and carcinoma in the forestomach.

The most recent study, conducted by NCI (Lijinksi *et al*, 1986), reported that styrene oxide was given by gavage at doses of 275 and 550 to F344 rats and at doses of 375 and 750 to B6C3F1 mice three times /wk for up to 104 wk. A high incidence of lesions indicative of irritation was reported, along with tumours in the forestomach of both species.

7.6.6 Dermal Studies of Styrene Oxide

In two dermal carcinogenicity studies, styrene oxide was applied to the skin three times /wk as 5% or 10% solutions in acetone or benzene. There were no increases in skin tumours in either C3H or Swiss-Millerton mice (Weil *et al*, 1963; Van Duuren, 1963).

7.6.7 Significance of Styrene Oxide Long-Term Studies

Long-term oral studies of styrene oxide have consistently shown a high incidence of tumours in the forestomach in rodents given high doses. The response in the rodent forestomach has been associated with intense chronic irritation at the site of application, and there has been

no indication of systemic oncogenic effects related to treatment with styrene oxide. The absence of an oncogenic response at the site of dermal application indicates that styrene oxide is not a potent genotoxic carcinogen when given under non-irritating conditions. This suggests that chronic irritation may be an important factor in the development of forestomach tumours in oral studies; the low degree of forestomach DNA-binding reported by Cantoreggi and Lutz (1992) further supports this. They suggest that the styrene oxide induced forestomach tumours were probably the result of strong tumour promotion by high-dose cytotoxicity followed by regenerative hyperplasia.

In general, the relevance of rodent forestomach tumours for human risk estimations is considered controversial, as discussed in recent reviews (Wester and Kroes, 1988 and Frederick and Chang-Mateu, 1990). Squamous cell carcinomas of the forestomach have been observed in many long-term dietary or gavage studies with rodents. Discrimination between DNA binding and cell division as mechanisms of action may be very important since a non-linear dose-response curve (if not a threshold) is likely to exist in instances were irritation, toxicity and regenerative hyperplasia are primary effects. Risk estimations are complicated by the absence of a homologue in man for the rodent forestomach, making it difficult to understand the mechanism of action. The development of forestomach tumours in styrene oxide gavage studies is of even greater uncertainty.

7.6.8 Summary of Animal Carcinogenicity Studies

In their review McConnell and Swenberg (1993) concluded that there was no convincing evidence of carcinogenic activity for styrene even though it has been studied using several species and strains of rodent, several routes of exposure (i.e. inhalation, gavage, drinking water, and intraperitoneal and subcutaneous injections). There was likewise no evidence of carcinogenic response when styrene was administered with β-nitrostyrene. The reviewers did however criticise the available studies as having deficiencies including design, conduct and interpretation. While it must be accepted that none of the studies may be ideal nevertheless the overall weight-of-the-evidence does not indicate that styrene is a carcinogen. Because of the deficiencies the styrene industry is currently undertaking a two year inhalation study in the rat (report expected 1995) and in the mouse.

7.7 HUMAN - EPIDEMIOLOGY

Human epidemiology studies and long-term animal bioassays are generally regarded as the most definitive means of assessing the carcinogenic potential of a chemical substance. Such data, when available should provide good information on exposure, latency, and potential confounders. Both IARC and EPA, two of the world's authorities on evaluating and classifying carcinogens, have taken this position.

The styrene epidemiological data base is extensive, including cohort mortality studies of workers employed in either the reinforced plastics manufacturing industry, in styrene monomer, polymer or styrene-butadiene rubber manufacturing operations e.g. Coggon *et al* (1987), Hodgson *et al* (1985), Matanoski *et al* (1987), Meinhardt *et al* (1982), Nicholson *et al* (1978), Okun *et al* (1985), Ott *et al* (1980) and Wong (1991) also referred to as the EHA

(Environmental Health Associates Inc.) Report. An in-depth, critical assessment of these studies, including nearly 50,000 workers during the time period from 1940 to 1986 - has been performed by Bond *et al* (1991). In addition, two of the cohorts have been updated Bond *et al* (1992) and Wong *et al* (1994) or reanalysed Santos-Burgoa *et al*, (1992); IARC has completed a historical cohort study involving 40,683 workers employed in the reinforced plastics industry.

The reason why several of the epidemiological surveys have concentrated on the reinforced plastics industry is because this is where the highest exposures occur. Okun *et al* (1985) cited average ambient styrene concentrations of 72 ppm at one plant, with some jobs receiving mean exposures over 100 ppm. Most of the work force in the study by Coggon *et al* (1987) were estimated to have had recent exposures from 40-100 ppm. Ahlmark (1978) estimated exposures in Swedish reinforced plastics industry operations around 1970 to be in the range of 250-350 ppm. The greatest contact with styrene is through the inhalation of vapours during spray operations and curing, and dermal contact during hand lamination with resin that contained about 40 percent styrene monomer.

Meinhardt *et al* (1982) cited much lower 8-h TWA concentrations for styrene in an SBR facility in 1977. The highest level recorded was 12 ppm and most samples were well below 1 ppm. Nicholson *et al* (1978) reported styrene exposures in 1974 to range from less than 1 ppm to 20 ppm. However, considerable changes have taken place in SBR processing, and higher exposures were likely historically, especially in batch polymerization processes before 1950.

Three studies (Hodgson and Jones, 1985; Meinhardt *et al*, 1982 and Ott *et al*, 1980), have suggested an association between styrene exposure and the occurrence of lymphatic and haematopoietic cancer (LHC). Ott *et al* (1980) found a statistically significant excess (living and dead) of lymphatic leukaemia among workers in the colorant blending, roll compounding and extrusion operations (5 observed, 0.26 expected). In the overall cohort leukaemia mortality was found in excess though not statistically significant (6 observed, 3.4 expected) with an absence of any lymphatic leukaemia among the groups with the highest styrene exposures. In a follow up study of this cohort (Bond *et al* 1992), in which mortality was updated by a further 11 years, the authors reported substantial deficits in mortality from all causes and total cancers was observed. The mortality data from leukaemia was slightly less than expected during the updated period, this is in contrast to the excess of lymphatic leukaemias seen in the first study. Small elevations in risks of other types of lymphatic cancer, particularly multiple myeloma persisted. The risks of the cancers was not however associated with intensity or duration of styrene exposure. Hodgson and Jones (1985) in a study of workers producing styrene monomer and polymers found a significant excess in lymphoma (believed to be non-Hodgkin's lymphoma), based on 3 observed deaths versus 0.6 expected. Meinhardt *et al* (1982) conducted a cohort study of two styrene-butadiene plants (designated Plant A and Plant B)which included 2,756 males with at least six months of employment. A non-significant excess of lymphatic and haematopoietic cancer (9 observed, 5.8 expected) was found in Plant A but not among the workers at Plant B. The majority of the excess at Plant A was due to leukaemia (5 observed 2.5 expected).

In contrast to the small increases in LHC seen in the studies described above Coggon *et al* (1987) reported only six cases of LHC among reinforced plastics workers when 14.9 were expected; the overall deficit was significant. The authors concluded that their *a priori* suspicions of styrene were not borne out. The study by Okun *et al* (1985) found no deaths from lymphatic and haematopoietic cancer in a large but relatively young cohort of reinforced plastics boat builders with limited follow-up. Similarly Nicholson *et al* (1978) found only one lymphoma and one leukaemia case, and concluded that while their data were not definitive, the environmental risk from styrene was likely "not extraordinary". A study of LHC in styrene-butadiene polymerization workers (Santos-Burgoa *et al*, 1992) suggested that there was not a significantly increased risk associated with exposure to styrene.

Wong (1991) published the findings of the EHA study which examined mortality in 15,908 male and female workers employed in the reinforced plastics industry in the US. The total numbers of deaths (499) was identical to the number expected. No significant mortality increase in cancer for all sites or from any specific site was found. In particular no increased mortality from cancer of the lymphatic and haematopoietic tissues was detected i.e.leukaemia deaths (5 observed vs. 4.8 expected) and 9 total LHC deaths (versus 12.3 expected). An excess of lung cancer, was seen among workers at plants using "hot processes", Standard Mortality Ratio (SMR) of 177 . A subsequent nested case-control study of respiratory cancer failed to find any association between respiratory cancer and styrene exposure; a significant association between lung cancer and cigarette smoking was found.

A large study by Kolstad *et al*, (1993) examined 552 companies in Denmark assumed to have produced reinforced plastics including records on 64,000 individuals employed between 1964 to 1988. Three quarters of the companies employed less than 20 people and less than half were still operational in 1988. The population, followed for cancer incidence from 1965 to 1989, showed no excess of cancer as compared with the national average. The male employees did however show a very small increased risk of malignant neoplasms of the lymphatic and haematopoietic tissues (SMR 1.2, CI 1.0-1.4); no excess was seen in females. An assessment of whether or not the 552 companies originally selected were in fact associated with the reinforced plastics industry (based on information from suppliers and questionnaires to employees) indicated that 386 companies could be positively identified as being involved in the production of reinforced plastics, 82 companies were totally unknown and 84 had never produced reinforced plastics. Men employed in the 386 companies, positively identified with the reinforced plastics industry, showed an SMR of cancers of the lymphatic and haematopoietic tissues of 1.3 - no information is provided whether or not this is significantly different from background levels or what happens if sexes are combined. Of the remaining companies the 84 with no known activities in the reinforced plastics business the risk ratio was unity (i.e. SMR 1.0) while the highest risk (SMR 1.9) was found in companies unknown to dealers presumably also not associated with the reinforced plastics industry. As this investigation, to date, has only been presented as a poster and an abstract it is difficult to have a good understanding of the data, the analyses performed and the results obtained. The authors suggest that the data indicate a small increased risk of malignant neoplasms of the lymphatic and haematopoietic tissues in employees in the reinforced plastics industry.

A historical cohort study undertaken by IARC including a total of 40,683 subjects comprising 8 cohorts from six countries has been reported by Kogevinas *et al* (1994). The population was divided into exposure groups distinguished on the basis of exposure measurements and type of work. For example laminate workers constituted the most heavily exposed group while maintenance and fork-lift truck drivers had lower exposures. Workers not exposed to styrene were clerical workers or those infrequently exposed to styrene. The study indicated a general decrease in styrene exposure in most of the countries with the exception of Finland. While exposures in general had dropped from levels above 200 ppm in the 60's to about 50 ppm by the 90's in Finland contemporary exposure levels still averaged about 100 ppm similar to levels measured in the mid 70's. The results show that among the exposed workers no increased risk was observed for mortality and no increased risk was observed for cancer at any site. An examination of the occurrence of lymphoma and leukaemia showed no association between the incidence of these forms of cancer with either duration or level of exposure to styrene. Among the exposed workers, especially with more than one year of exposure, mortality from lymphomas and leukaemia's increased with time after first exposure even though there was no consistent pattern in risk with length of employment. Interpretations of such data is difficult since in general mortality rates from cancer are generally lower than normal but then increase gradually with time since first exposure. The authors suggest that no firm conclusion will be reached until the cumulative exposure to styrene and cancer incidence data have been obtained fully and analysed. Until then they conclude that the findings are inadequate to exclude the possibility that styrene may cause leukaemia and lymphoma. The study's null hypothesis is that there is no increase in deaths from leukaemia and haematopoietic cancers associated with exposure to styrene during the study time period. Based on the data collected and the analyses performed currently this null hypothesis can not be rejected.

The follow up on the cohort studied by EHA was reported by Wong *et al* (1994). The updated mortality data for a further 12 years bring the number of deaths up from 499, in the original study, to a total of 1,644. An important observation of this study was the lack of relationship between exposure to styrene and increased risk of death from lymphatic and haematopoietic cancers as well as leukaemia even though the study has the power to detect risks as small as 1.44 for all lymphatic and haematopoietic cancers and 1.74 for leukaemia. The study did however show significant increases in deaths from a number of causes including all cancers, lung cancer, kidney cancer, prostate cancer, cancer of female genital organs, hypertensive heart failure, certain non-malignant respiratory disease, motor vehicle accidents and homicides. However when the mortality patterns were examined for length of employment in the industry no upward trend was detected. In fact the mortality increased almost exclusively among workers employed in the reinforced plastics industry for no longer than 6 months to a year. The results of the study suggested that poor health or health habits and low socio-economic status, characteristic of short-term workers, might be responsible for the elevated mortalities. Previous studies have linked low socio-economic status to a variety of diseases including, lung cancer, cancer of the prostate, cancer of female genital organs , heart disease, hypertension and unintentional injury (Baquet *et al*, 1991, Kaplan *et al*, 1987).

Generally the evidence that styrene increases the incidence of cancer of lymphatic and haematopoietic tissue, or indeed any tissue, is extremely weak or non even existent. This is supported by the conclusion reached by Richardson *et al* (1992) who after examining occupational risk factors for acute leukaemia found no relationship between styrene exposure and an increased risk of leukaemia. Any risk associated with occupation in the reinforced plastics industry (which at worst must be extremely small) most certainly occurred in earlier years when the exposure to a variety of chemicals was little documented and most probably considerably higher.

In addition to the studies described above two other reports occur in the literature which, although providing some information on styrene exposure have not been considered to be useful for assessing potential styrene carcinogenicity. A report by Ahlmark (1978) reported no excess of cancer incidence. The follow up period was too short to detect any increase and the report is only useful in giving a demographic description of a cohort targeted for possible future investigation. Similarly the report by Frentzel-Beyeme (1978) of a styrene-polystyrene production plant is of little value since the report only provided comparisons on relative proportions of causes of death rather than rate, i.e. it is a proportional mortality study. The study was also criticised by the WHO (1983) for not providing mortality rates of reference populations and having an incomplete follow up.

A third study reported by Bloemen *et al* (1992) was initiated when two men ,who had worked in the polystyrene manufacturing and colouring and compounding plant (PMCC) located in the Terneuzen manufacturing site of the Dow Chemical Company in the Netherlands, were diagnosed as having renal cell carcinoma (RCC). Two other cases were previously known to exist (one of whom also worked in PMCC the other only limited involvement). An ultrasound screening programme of 711 employees directly or indirectly involved in PMCC was undertaken together with an epidemiological study to identify possible risk factors. In summary the ultrasound programme failed to find any more cases while the epidemiological survey failed to find a risk factor to explain the cluster although two additional historical cases of RCC were identified both of which had no links with PMCC. Five of the finally identified six cases were life-long residents of the rather isolated Zeeuwsch-Vlaanderen region and while the relative risk was statistically significant it is inconsistent with cancer incidence statistics and assumed to be circumstantial. The medical surveillance programme continues to monitor populations for any indication of occupationally related illness. It is probable that if any extraordinary risk of styrene exposure and renal carcinoma existed this would have been identified in other more extensive epidemiological surveys including populations having had far higher exposures to styrene.

7.8 OVERALL CANCER RISK ASSESSMENTS

In a review of the available animal carcinogenicity studies McConnel and Swenberg (1993) concluded that there was no convincing evidence of carcinogenic activity of styrene in experimental studies with rodents. The only positive evidence stems from a gavage study showing forestomach tumours. The relevance of this study is limited because: a) the exposure route is not relevant to man, b) no neoplasms were found at sites distant from the site of exposure and c) irritating xenobiotics are known to cause such local neoplasms. None

of the other animal data show any real evidence for styrene to be a carcinogenic hazard.

Similarly the human data have failed to demonstrate styrene to represent a carcinogenic hazard.

Although there are no data for styrene demonstrating a carcinogenic hazard, a number of investigators have attempted to provide some sort of "quantitative" assessment of carcinogenic risk based on the fact that styrene is metabolised via styrene oxide SO. Since concern is related to the "internal dose" of SO rather than the "applied dose" of styrene kinetic and metabolic data have been generated to develop a physiologically based-pharmacokinetic (PB-PK) model for rats, mice and humans after inhalation. Using this model the ECETOC Styrene Task Force (ECETOC Technical Report No. 52, 1992) compared the internal dose of SO in humans exposed to 50 ppm styrene with that occurring in animals as exposed in the various oncogenicity studies. This approach shows that the internal dose of SO in rodents at exposure levels without an oncogenic response can be up to 400 times higher than that in humans exposed to 50 ppm of styrene. This "semiquantitative margin of safety" approach of the ECETOC Styrene Task Force (1992) also shows that the levels of SO in rodents showing no evidence of cytogenetic damage (Preston and Abernathy, 1993) can be two to three orders of magnitude higher than the internal, low levels of SO occurring in humans working with styrene at current recommended exposure levels.

The PB-PK model has also been used by Filser *et al*, (1993) to provide an estimate of possible carcinogenic risk. In principle, they calculated a mean lifetime exposure to the SO and limited this to a statistically derived upper bound limit for a possible tumour incidence in the most relevant animal experiments. It was necessary to use a statistically derived upper bound limit for cancer because in most studies an actual increase in tumour incidence was not found. Using this approach the "tumour probability" (maximum possible human risk) was estimated to be in the range of 1.7×10^{-5} and 3.6×10^{-5} for a lifetime exposure at 20 ppm.

A second approach used the haemoglobin adduct formation of SO and ethylene oxide. Assuming 1) the existence of a relationship between carcinogenic risk and haemoglobin adduct formation and 2) the ratio of this relationship to be identical for both ethylene oxide and styrene oxide, the cancinogenic risk estimated was 5.9×10^{5} for a lifetime occupational exposure to 20 ppm (Filser *et al*, 1993).

Cantoreggi and Lutz (1993) and Filser *et al*, (1993) estimated a "theoretical risk" posed by styrene using the Covalent Binding Index calculated from the measured covalent binding of radiolabelled styrene to DNA *in vivo*. After inhalation very low adduct levels of 0.1 CBI were found (near the limit of detection) in mouse liver and in some of the rat lung samples. Using these minute amounts of binding the authors estimated a D_{50} theoretical dose assumed to produce a 50% tumour incidence rate of approximately 100 g/kg/d and a theoretical cancer risk in humans at 20 ppm of 1×10^{5}. In contrast to the risk assessments mentioned above, this latter approach does not take into account any inter-species differences in kinetics and metabolism of styrene and SO.

Taking together all animal, biochemical and epidemiological data, a carcinogenic hazard of styrene has not been observed. The mechanistic data indicate that if styrene is considered to represent a carcinogenic hazard through its metabolism (and this is an assumption not supported by available data) the related risk must be still very small when the workplace exposure limits applied today.

7.9 DEVELOPMENTAL AND REPRODUCTIVE TOXICOLOGY

There is little indication that styrene exerts any specific developmental or reproductive toxicity. Animal studies in four different species indicate that styrene is not a selective developmental toxicant. There is no evidence of developmental effects in humans. Styrene has not exerted effects upon rat reproduction or mouse sperm morphology. The available information in humans is inadequate to define possible reproductive effects due to styrene exposure. The literature for styrene reproductive and developmental toxicity has been thoroughly reviewed by Brown (1991).

Weight-of-the-evidence indicates that styrene is not a teratogen, or able to selectively affect the conceptus although well-designed, and well-reported developmental toxicity studies are lacking. There was no evidence of external or soft tissue effects in the progeny of rats exposed to 300 ppm or 600 ppm by inhalation for 7 h/d, d 6 through 15 of gestation. While at 300 ppm a higher incidence of skeletal variants (primarily delayed ossification) was observed, the incidence was within historical control range (Murray *et al*, 1978). At both doses

a reduction in maternal weight gain and food consumption was reported. Vergieva *et al*, 1979, exposed rats to styrene at 94 or 163 ppm for 4 h/d, 5 d/wk, throughout most of gestation. No adverse effects on pre- or post-implantationS were seen nor was there any increase in malformations. Rabbits exposed to either 300 ppm or 600 ppm exhibited no maternal toxicity, and only a slight increase in skeletal variants which were within historical control (Murray, *et al*, 1978). A styrene developmental toxicity study with mice and Chinese hamsters was reported by Kankaanpaa *et al* (1980). Mice exposed to 250 ppm styrene for 6 h/d, gestation days 6-16, showed an increased incidence of dead and resorbed foetuses and a small increase in skeletal malformations. However these effects were considered of low significance. No visceral examination was made, and maternal toxicity was not described. The statistical test being inadequate it can be concluded that no real effect upon either teratogenicity or lethality occurred. Chinese hamsters were exposed to styrene at concentrations of 300, 500, 750, or 1,000 ppm for 6 h/d, gestation days 6-18. There were no differences between the controls and groups treated with 300-750 ppm. A significant increase in dead and resorbing fetuses was reported at 1,000 ppm. Unfortunately as no objective measures of maternal toxicity were made the reported increase in lethality is difficult to interpret.

A number of investigations have been undertaken in which animals received oral doses of styrene. For example Daston *et al* (1991) administered styrene by gavage (dose 300 mg/kg bw) to rats on gestation day 11. While the treatment caused a decrease in food consumption and maternal weight change it did not adversely influence any developmental parameter. Styrene administered to rats at doses of 180 or 300 mg/kg/d, gestation days 6 to 15,

diminished maternal weight gain and food consumption, but had no effect upon development (Murray *et al* 1978). Srivastava *et al* (1989a) reported that rats treated with styrene at 400 mg/kg/d, dosing through gestation days 6 to 15, showed evidence of maternal toxicicity with associated fetal resorptions and decreased fetal weight. Treatment at 250 mg/kg/d was without effect.

Pups of dams exposed either by inhalation (Vergieva *et al*, 1979) by gavage (Zaidi *et al*, 1985), or in drinking water (Beliles *et al*, 1985) showed no effects on growth or behaviour. Vergieva *et al*, 1979 reported an increase in the number of erythrocytes after one month in both dams and pups in the 47 ppm exposure group (not measured in the 163 ppm group). How to explain this result in regard of reproductive toxicity is difficult without further details. Khanna *et al* (1991) found no postnatal behavioural changes in rat pups exposed to styrene at 100 mg/kg/day. Styrene exposures have not been associated with adverse effects on postnatal function. The available studies unfortunately are limited by small sample sizes.

Investigations in rodents to examine whether exposure to styrene causes damage to gonadal tissues were negative. Beliles *et al* (1985), administered styrene to rats in their drinking water at either 125 or 250 ppm (maximum concentration of approximately 21 mg/kg/d). The rats were exposed for two years during which time males and females were selected randomly for breeding after which they were returned to the study. The F1 generation was also exposed to styrene in the drinking water and around day 110 were mated to produce an F2 generation. An F3 generation was subsequently derived. The treatment produced no gross or histopathological changes ans no evidence of carcinogenicity in any of the animals. No testicular effects were found in male rats exposed via inhalation for ninety days at up to 1500 ppm (personal communication, SIRC). Chronic inhalation exposure of male rats to either 600 ppm or 1,000 ppm did not affect spermatogenesis or morphology, although focal microscopic lesions, attributed to physical trauma, were seen in the testes at both doses (Jersey, *et al*, 1978). Styrene administered to male mice at 150 ppm or 300 ppm, 6 h/d for 5 d, had no effect upon sperm counts, testicular morphology, or the number of abnormal sperm when evaluated three and five weeks post-exposure (Salomaa, *et al*, 1985). Srivastava, *et al* (1989) did report oral administration of styrene, 400 mg/kg, 6 d/week, for 60 d, causing a reduction in epididymal sperm count and degenerative changes in the testes. This report is opposite to the findings of the majority of studies.

In his review of the available data on styrene and reproductive toxicology Brown (1991) reported that many of the studies on the developmental and reproductive toxicity of styrene are of low quality and should be given little or no consideration. Thus the animal studies reporting either developmental (Ragule 1974; Efremenko and Malakhovskii, 1976; Ponomarkov and Tomatis, 1978; Vergieva and Zaikov, 1980) or reproductive (Bondarevskaya 1957; Izyumova *et al*, 1971; Zlobina *et al*, 1975; Bakhtizina and Khakimov 1982; Bakhtizina *et al* 1983; Bakhtizina and Popucshiev 1981) effects, were considered incomplete and of inconsistent quality and not useful in any evaluation.

Brown (1991) also considers human evidence inadequate. In humans, a number of case-referent results have been published, but the overall numbers are too small to warrant any conclusions. There is a suggestion that solvent exposure in general, may be associated with

central nervous system defects and oral cleft defects, but no specific implication for styrene (Holmberg, 1977, 1978, 1979; Holmberg and Nurminen, 1980; Holmberg *et al*, 1982; Kurppa, *et al*, 1983; Holmberg *et al*, 1986; Harkonen and Holmberg, 1982; Harkonen *et al* 1984; Hemminki, *et al*, 1984; Alborg *et al*, 1987). In addition, no specific association is evident for styrene exposure and spontaneous abortions (Hemminki, *et al*, 1980; Lindbohm, *et al* 1985).

Three studies have been reported regarding female exposure to styrene at the workplace. For gestation length, birth weight, and menstrual cycles no statistical association was shown between exposure and the endpoints. Sample size however limit the power of these studies (Lemasters *et al*, 1985a; 1985b; 1989; Samuels *et al*, 1985). Harkonen and Holmberg (1982) observed no differences between solvent-exposed (including styrene) women and menstrual irregularities. Other studies indicating styrene exposure to be associated with menstrual dysfunction failed to report control data and/or confounding factors (Zlobina *et al* 1974; Loseva *et al*, 1983; Zlobina *et al*, 1975).

There are two studies published on male reproductive effects. Neshkov and Nosko (1976), reported that exposed men had sexual performance problems, reduced ejaculate volume, lowered sperm count, increased sperm abnormalities, altered steroid metabolism, and psychological problems. The subjects had multiple chemicals exposures. Relative exposures were however not provided. It is not possible to draw any conclusion from this study. A Danish study (Jelnes, 1988) suggested that styrene exposure may increase sperm head abnormalities, as well as have effects upon other sperm parameters. This study was compromised by the source of the control group (semen samples from men visiting an infertility clinic).

7.10 NEUROTOXICITY

7.10.1 Studies with Human Volunteers under Controlled Conditions

There have been several investigations of the effects of a single exposure to styrene using human volunteers under controlled conditions.

In an early study by Carpenter *et al* (1944), two human volunteers were exposed to 800 ppm styrene for 4 h. The subjects reported listlessness, drowsiness, and impairment of balance during exposure. After termination of the exposure, slight muscle weakness and unsteadiness, as well as inertia and depression were experienced. There was also an impairment in the performance of two behavioral tests after the exposure.

Stewart *et al* (1968) conducted a study to evaluate the effects of a single 7 h exposure to 100 ppm styrene in several male human volunteers, and a study to determine the concentration at which symptoms of neurological impairment appear. This latter objective was evaluated using I h exposures to styrene concentrations of 50, 117, 216, or 376 ppm. During the exposure period, various psychomotor tests were performed. No adverse effects other than very mild and transient eye and throat irritation were noted in subjects exposed to 100 ppm styrene for 7 h; some difficulty was occasionally reported in performing a Romberg test (a test designed to measure coordination and balance), but this was believed not to be

significant. The results of the 1 h exposures indicated that mild irritant effects first occurred at 216 ppm, with one subject reporting marked irritation at this concentration. More pronounced nasal irritation and mild eye irritation was noted at 375 ppm. Impaired performance in the psychomotor tests was noted at 375 ppm, but no significant effects were noted in these tests at 216 ppm or lower.

In a study involving 12 human volunteers, Gamberale and Hultengren (1974) exposed the subjects to styrene concentrations of 50, 150, 250 and 350 ppm in 30 minute consecutive steps. Various psychological/psychomotor tests were performed during the exposure period. The only statistically significant effect was a slight impairment in reaction time at 350 ppm . Subjective evaluations suggested that subjects exposed to the higher concentrations were more tense, but the relevance of this observation is questionable since they were aware of their exposure to styrene. The authors concluded that styrene exposure concentrations around 50 ppm did not have unfavourable effects on human performance.

Oltramare *et al* (1974) reported studies under controlled conditions in which small groups of up to six volunteers were exposed for periods of 1 to 3 h to styrene concentrations of 50, 100 or 200 ppm. Subjective symptoms including headache, sleepiness, fatigue, and difficulty in concentrating were noted at all exposure concentrations, but were more prevalent at the two higher exposure concentrations. Decrements in psychometric tests (visual reaction time, audiovisual reaction time) were also reported at all exposure concentrations, with more pronounced effects at the two higher exposure levels. The observations at 50 ppm in this study are not consistent with the results of other chamber studies. The authors mention that it was impossible for them to prevent variations in the exposures, and it is possible that the subjects experienced high peak exposures. Because of the small numbers of subjects and uncertainties about exposures and success in blinding, the results of this study need to be interpreted in the context of other chamber studies.

Hake *et al* (1982) evaluated the responses of 10 male and 8 female volunteers exposed experimentally to different concentrations of styrene up to 125 ppm for 7.5 h on at least 4 d every week for 4 wk. Blood, breath and urinary metabolites were evaluated for purposes of monitoring repeated daily exposures. Analysis of styrene in alveolar breath samples obtained 15 minutes post exposure was found to provide an excellent measure of the magnitude of the day's exposure. Measurements of blood styrene concentration and urinary metabolites were found to be of limited value for monitoring due to the large variability and the difficulty of obtaining and analysing samples. Clinical examinations revealed no styrene effects. Electroencephalographic (EEG) responses were evaluated for 6 subjects. The EEG's were highly variable, but the authors concluded that for 3 of the 6 subjects there were no EEG changes during the study; for the remaining 3 subjects there was a tendency towards slower frequency and higher amplitude as the study progressed. Complaints of headaches, dizziness and nausea were reported by some subjects exposed to 100 or 125 ppm. Cognitive tests showed no effects attributable to the styrene exposure.

7.10.2 Studies in Workers Occupationally Exposed to Styrene

There are numerous reports on workers exposed to styrene. The value of these studies is frequently limited by the lack of reliable exposure information or the failure to compare results to a properly matched control group.

Hruba *et al* (1975) reported the results of a cross-sectional study of 122 Czechoslovakian workers (27 men and 95 women), but no data were available on the styrene exposure concentrations. Approximately 30% of the subjects complained of sleep disorders and headaches, and an increased incidence of electroencephalograph (EEG) abnormalities was reported. The results of this study are difficult to evaluate because of the lack of exposure information, and the absence of a control group with blind reading of the EEG's.

Klimkova-Deutschova *et al* (1962) also reported complaints of headache, fatigue and EEG abnormalities in a group of 35 Czechoslovakian workers exposed to styrene. However, there was no exposure information, and the report did not allow any estimate of the prevalence of the effects. In a study carried out in the USSR, symptoms such as headaches, nausea, dizziness and unsteadiness were reported in a group of 70 workers exposed to styrene over a three year period; no information was provided regarding the extent of the styrene exposures (Dzyuba *et al*, 1972).

An additional field study involving 17 workers exposed to styrene and 27 aged matched controls (occupation unknown) was reported by Gotell *et al* (1972). Styrene exposures (time-weighted average) during the workshift ranged from 17 ppm to 292 ppm, with peaks up to 1500 ppm. Neurological examinations were normal except for two subjects with slightly unsteady Romberg tests before, but not after, exposure (concentration unspecified). Reaction times were slower for the exposed group than for the controls on the morning before exposure. At the end of the work shift, the reaction times of the exposed group improved slightly, but were still lower than for controls. In a subgroup exposed to less than 150 ppm, the reaction time in the morning was only slightly slower than for controls, and at the end of the work shift the reaction times of this group were almost identical to controls.

Abnormalities in EEG's were also reported in a group of Polish workers involved in the manufacture of plastic lifeboats (Dolmierski *et al*, 1976). Subjective symptoms, including fatigue, weakness and drowsiness, were noted. Two groups of workers were evaluated, grouped according to age and duration of exposure. It was not clear if the workers were a random sample or the complete population from the two factories. There was no control group and most importantly the investigators were not blind to the hypothesis under investigation. The exposures were highly uncertain, but it was stated that exposures were as high as 72 ppm. No information was given regarding the measurements on which these estimates were based. In view of these limitations, the results of the study cannot be taken as anything more than suggestive.

Gamberale *et al* (1976) investigated the effects of occupational exposure to styrene on reaction time. The study involved a total of 106 workers with an average duration of exposure to styrene of 2.7 years. Limited information was obtained regarding the styrene

exposure concentrations. Exposures were monitored over one work shift. It is not explained how representative these measurements were for other exposure times. During the shift monitored, mean styrene concentrations of 16-104 ppm were recorded for resin workers in various plants. Assemblers were exposed to concentrations as high as 280 ppm detected in one plant. The results of the study indicated that the styrene workers had longer reaction times increasing more during the workshift than the control group. It was not possible to assess a dose-response relationship from the data. Insufficient information was provided to draw any clear conclusions about a threshold concentration for the impairment of reaction time.

A pilot study by Rosen *et al* (1978) was conducted to investigate neurophysiological effects in small groups of workers from three factories in Sweden. The mean exposure styrene concentrations determined by personal sampling at the three plants were reported to be 125, 47 and 5 ppm, respectively. The questionnaire used indicated an inreased incidence of various symptoms (tiredness, giddiness, memory loss) in the group exposed to 125 ppm. Medical examinations of the workers in this group showed signs of irritation of the conjunctiva and throat but no signs of polyneuropathy. No differences in motor nerve conduction velocity between any of the groups were identified. There was some indication of mild sensory neuropathy in the styrene exposed workers, suggested by decreased duration of sensory amplitude potential. However, the observed effects were comparable in the high and low exposure groups, and it is therefore doubtful that the effects were related to styrene exposure. No substantial differences in EEG's were noted.

Lilis *et al* (1978) reported the results of physical examinations of nearly 500 U.S. workers involved in the manufacture and polymerisation of styrene. A companion survey reported by Lorimer *et al* (1976; 1978) focused on job-related symptom histories in the same group of workers. No exposure data were presented (either in ppm or concentration of urinary metabolites), but the workers were somehow judged to be currently in either a low styrene exposure group or a relatively higher exposure group. The high exposures were stated to be in the order of 20 ppm and the low exposures being less than 1 ppm. These groupings apparently were made after discussions with the workers and management. Hypoesthesia (touch and pain) in the lower extremities was reported to increase from 4.1% to 8.5% with increasing styrene exposure concentration, and a similar trend was reported for hypoactive deep tendon reflexes in workers with more than 20 years of exposure. There was a tendency (without statistical significance) for decreased peroneal nerve conduction velocity with duration of exposure in both the high and low exposure groups, and radial nerve conduction velocity was reported to be slowed in 19% of those exposed for more than 7 years. A higher incidence of prenarcotic symptoms (lightheadedness, dizziness, headaches) was reported in the "high" exposure group than in the "low" exposure group. Interpretation of this study is seriously limited by the absence of control group comparisons, and the failure to correct for age-related changes. Due to its limitations, it is impossible to draw any conclusions from this study about the potential neurotoxic effects of styrene.

The results of a cross-sectional neurological survey of nearly 100 workers exposed to styrene in 24 different polyester plants in Finland have been extensively reported (Seppalainen and Harkonen, 1976; Lindstrom *et al*, 1976; Harkonen,1977; Harkonen *et al*, 1978). No atmospheric styrene exposure information was given, but end-of-shift urinary mandelic acid measurements were made on a different week day for five wk prior to the examinations. The mean urinary mandelic acid concentration was reported to be about 800 mg/l, with measurements ranging from 7-4715 mg/l. EEG's were considered to be abnormal in 24 individuals; 8 with diffuse theta activity, 14 with localized slow waves, and two with bilateral spike and wave discharges. No control group comparisons were made in these studies, but the incidence of abnormalities was related to urinary mandelic acid concentrations. Peripheral nerve conduction velocity measurements in 40 workers judged to have the highest styrene exposure showed no reduction in mean conduction velocity values in comparison to control group data previously obtained by the same investigators; no concurrent control group data was provided. The authors concluded that the mild EEG abnormalities were confined to those individuals with urinary mandelic acid levels greater than 700 mg/l; this was estimated to represent an equivalent 8-hr TWA exposure to 30 ppm, but the actual exposure cannot be inferred with any degree of certainty from urinary metabolite concentrations (see Toxicokinetics section). The authors also concluded there was no evidence of exposure-related changes in nerve conduction velocities. In addition to the neurological assessments, each individual was subjected to an extensive battery of psychological or behavioral tests. The results for the styrene workers were compared to a control group of 43 concrete reinforcement workers stated to be of similar age and educational level. The authors concluded that there were very few differences between the styrene workers and the control group. However, some impairment in two tests for visuomotor accuracy and in one test of psychomotor performance was observed in workers with post-shift MA levels of 1200 mg/l or higher.

The results of psychological function tests on a very small group (7 males) of workers in a Swedish boat factory were reported by Kjellberg *et al* (1979). Air sampling measurements were taken throughout the workday; it is not known how representative these exposures are of other work shifts. This study was very unusual in that the workers were subjected to needle biopsies of fatty tissue for measurements of styrene concentration in the subcutaneous tissue; this indicates the workers to be highly motivated and not acted blind in the sence of the study. A control group of 7 workers was matched for age, height and weight, but there was no consideration given to other factors such as smoking. The TWA styrene concentrations for the workers ranged between 3-14 ppm, and the styrene adipose tissue concentrations were in the range of 3.1-13.6 mg/kg. Psychological tests showed no significant differences between the control and exposed workers, but there were small differences in the reaction times of the two groups. The impaired reaction times of the exposed workers did not correlate with estimated styrene uptake unless account was taken of the amount of adipose tissue present; those with the least adipose tissue had the greatest impairment. There was some improvement in the reaction times of three individuals when measured 4-7 d post exposure. The authors therefore concluded that the effects on reaction time were at least partially reversible. In view of the small number of individuals involved and the lack of data on factors such as smoking which are known to affect reaction times, no firm conclusions can be drawn from this report.

Cherry *et al* (1980) investigated the acute behavioral effects of styrene in a group of 27 boat builders. The results for these workers were compared to a control group of 27 men employed at the factory who were not expected to be exposed to styrene. Little information was given about the environmental styrene exposures. The mean styrene exposure concentration during one work shift was reported to be 92±46 ppm, with the exposures being greater in the morning (117±63 ppm) than in the afternoon (52±35 ppm). It is not known whether these exposures are typical of other work days. Urinary mandelic acid (MA) concentrations were measured, but not at the end of shift; this makes comparisons with MA measurements in other studies difficult. Styrene blood concentrations were measured at the end of shift for both exposed and comparison groups. The exposed workers were divided into two subgroups on the basis on styrene blood concentration (those with styrene blood levels above and below 5.4 µmole/l), in order to assess dose-response relationships. There was very poor correlation between the biological and environmental exposure measurements, however. The correlation coefficient was only 0.44 for the blood styrene and average environmental styrene exposure measurements, and there was no correlation between blood styrene and urinary mandelic acid concentrations. The workers with the high styrene blood concentrations reported more mental and physical tiredness, and this group also had consistently slow reaction times both in the morning and in the afternoon. The reaction times of the exposed group were unexpectedly slower than for the control group at the start of the shift, and then either improved or remained the same during the course of the work shift. The authors noted that prior knowledge of the general purpose of the study might well have influenced the verbal responses of the exposed group, but there was nevertheless a correlation between styrene blood concentration and change in mood. No significant differences were noted between the exposed and control groups in any of the other tests that were conducted. The results of the study suggest that occupational exposure to styrene concentrations in the range of 100 ppm may be associated with feelings of increased tiredness, and a slight impairment in reaction time. There was no consideration of the potential influence of age or smoking, both of which can affect reaction times. Interpretation of the results is therefore difficult, and no firm conclusions can be drawn from the study.

Additional studies by Cherry *et al* (1981) were conducted to further evaluate the pre-shift slowing in reaction time described in their previous study. Previously reported results (Cherry *et al*, 1980) were reevaluated using urinary mandelic acid (MA) concentrations in the earliest available sample in relation to the start of shift reaction time. Reaction times and early morning urinary MA concentrations were also measured in a small group (17 men) of workers not previously examined. Some of these workers (12) were re-investigated after a 16-day holiday during which there was presumably no styrene exposures. Reaction times were also evaluated for 8 men under conditions of high exposure, and then again several months later when exposures had been substantially reduced. Little environmental exposure information is presented, but the mean styrene exposure was approximately 23 ppm at the time of follow-up compared to about 100 ppm in the first study. It is not known how representative these shift exposures are to other days. Significant correlations were reported between the start of shift reaction times and urinary MA concentrations. In the follow-up of workers with reduced exposures, only 3 of 8 had improved reaction time scores while the remaining 5 workers had no change in reaction time and very little change in mean urinary MA concentrations. There was no improvement in the reaction times of workers after the holiday

period; 2 of these workers were found to have MA in their urine prior to new exposures to styrene. At the end of first day of work after the holiday, reaction time was negatively correlated with MA in an end of shift urine sample (i.e., those with least MA had the slowest reaction times). As in the first study, there is no discussion of any relation between reaction time and age. Due to the small numbers of workers involved, it is difficult to draw any firm conclusions from these studies.

More recent studies by Cherry and Gautrin (1990) involved 70 male workers from 4 factories, with mean age of 29 years and with styrene exposures from 30 d to more that 20 years. Reaction time and nerve conduction velocity were measured on the Monday after a weekend with no known styrene exposure, and the results were related to concurrent styrene biological and environmental exposure measurements. Nerve conduction velocity was reevaluated after a lay off period of at least 67 d. Reaction time was also measured after a holiday break of 3 weeks. Environmental styrene exposure data was limited to measurements taken during one 8-h workshift. Detailed exposure data was given only for 59 individuals included in the analysis of nerve conduction velocity. Exposures to styrene were correlated with slowing of sensory but not motor nerve conduction velocity; these measurements were not corrected for temperature or age. No dose-response was found between slowing of conduction velocity and exposure to less than 50 ppm styrene. Reaction time scores were strongly correlated with age. The age adjusted reaction time score was not related to estimates of styrene exposure, but it was related to area under the excretion curve as well as the presence of MA in the urine on the morning the reaction time test was carried out. No correlation was found between symptoms reported and styrene exposure. The study demonstrated a relation between mild sensory slowing and styrene exposures of 100 ppm or greater (based on single work shift monitoring), and it confirmed an earlier finding of slower reaction times in workers who have urinary MA after a weekend break. The authors concluded that, although the numbers are small, the results indicate that the effects of styrene were reversible when exposures were discontinued.

Mutti *et al* (1984) evaluated several neuropsychological functions in workers exposed occupationally to styrene. The authors reported that the 50 styrene exposed workers and 50 matched controls differed from each other on seven out of eight neuropsychological tests applied. However, when the dose-response relationship is considered, only the tests for reaction time, the block design and digit symbols show some significant correlation with either intensity or duration of exposure as inferred from urinary mandelic acid (MA) and phenylglyoxylic acid (PGA). Moreover, when both duration and intensity of exposure is taken into consideration (as reflected by urinary MA and PGA), only the reaction time test remains significantly different. The actual styrene exposures in this study cannot be inferred with any degree of certainty from urinary metabolite concentrations. In addition, the study was a cross-sectional design (i.e., that is a study at a single point in time) which was potentially subject to confounding effects. Nothing is known about the occupations of the subjects in the control group except that they were manual workers. These subjects could have had quite varied skills that affected their job selection, and any differences between the groups could reflect differences in job skills. Prior knowledge among the styrene workers of the purpose of the study and the hypothesis tested could easily have affected the response of the subjects, whether consciously or subconsciously. The results of the study cannot be interpreted

because their was no attempt to evaluate the subjects over the course of time, and it is impossible to surmise whether the styrene workers before employment would have had the same scores as the controls on the neuropsychological tests applied.

In a small group of Swedish workers (12 men) exposed to 10-13 ppm styrene, Edling and Ekberg (1985) reported no adverse effects on reaction time, and no differences in subjectively reported symptoms between the exposed and reference groups. Urinary mandelic acid (MA) concentrations before work showed small negative correlations with reaction time. Correlations between styrene exposures during work with end of shift MA concentrations were likewise small, negative and non-significant.

Mackay *et al* (1986) measured reaction times in a group of 10 women exposed occupationally to styrene. Mandelic acid (MA) concentrations in end of shift urine samples were measured, but no styrene environmental measurements were carried out. The workers were divided into 3 groups; those with no MA (2 workers), those with MA less than 0.5 (3 workers), and those with greater than 0.5 mmole/mmole creatinine (5 workers). The pre-shift reaction time scores were paradoxically faster for the subgroup with the highest MA levels than for the others. Post-shift, those with no MA and those with low MA concentrations had improved reaction time scores in comparison to pre-shift values, while those with high MA values slowed down considerably. In view of the small number and the lack of styrene environmental measurements, no firm conclusions can be drawn from this study.

A longitudinal study was conducted by Triebig *et al* (1985), focusing on nerve conduction velocities in 11 German workers exposed occupationally to styrene. Styrene environmental measurements on three occasions over an approximate one year period indicated that the mean exposure was approximately 100 ppm. Clinical examinations showed no sign of peripheral neuropathy. The authors concluded that the nerve conduction velocity determinations did not reveal any indication of manifest or sub-clinical polyneuropathy. Conclusions to be drawn from the study are limited by the small number of individuals involved.

A subsequent study by Triebig *et al* (1989) included 36 men (ages 24 to 59 years) from four factories with a mean styrene exposure of 7 years. A comparison group of 20 people aged 22 to 55 years without exposure to styrene was selected from two companies. Styrene environmental measurements during the shifts indicated that the mean TWA for the exposed group was 18 ppm. The styrene exposures differed markedly between the factories, with one factory having a mean exposure of 136 ppm (range 48 to 251 ppm). The mean urinary mandelic acid (MA) concentration was reported to 0.06 g/l for the styrene workers on Monday morning including as high as 1.66 g/l. This suggests that some workers had either very high styrene exposures the previous week, or that clearance was very slow. At the end of the shift, the mean urinary MA concentration was 0.21 g/l, and the mean blood styrene concentration was 39 mg/dl. Prenarcotic symptoms of dizziness, nausea and headache during and after work were reported by four of eight workers exposed to concentrations over 100 ppm for the whole shift. The clinical examinations gave no indication of polyneuropathy or "psycho-organic syndrome". A battery of psychological tests showed no differences over the work shift between the styrene exposed workers and the unmatched controls. There was apparently no

attempt to assess any potential dose-response relationships by comparing results from workers with high styrene exposures to those from workers with lower styrene exposures. The authors concluded that there should be acute or chronic neurotoxic effects detectable on a group basis if styrene exposure concentrations do not exceed 100 ppm as an average for the work shift.

A small group of workers (21 males) exposed to styrene were evaluated for neuropsychiatric symptoms in a survey reported by Flodin *et al* (1989). The subjects in this study were evaluated on two occasions, one week after the closing of their place of employment due to bankruptcy and again several months later. Even though this was a longitudinal approach, there was unfortunately no time-matched appropriate control group. In a medical questionnaire, nine workers that were categorized as having a high styrene exposure (12 ppm) reported more subjective symptoms (e.g., headache, fatigue, personality changes, etc.) than a group of 8 workers placed into a low (6 ppm) styrene exposure category. The actual styrene exposures of these subjects is highly uncertain and was inferred from periodic measurements for different job categories during a period prior to the study. The authors did not report any attempt to rule out differences between the groups (type of work, work conditions, job satisfaction, etc.). The differences between symptoms reported one week and several months after the styrene exposures could have been accentuated by prior knowledge among the workers of the purpose of the study. Although the authors state that workers did not know about closing of their work facility at the time they were first reporting symptoms, it is possible that other factors (e.g., labour management relations, or deteriorating economic conditions) could have influenced the reporting. The subjective data presented in this study is therefore potentially confounded and is uninterpretable with regard to styrene exposures. A subsequent report by Moller *et al* (1990) involving the same group of workers is likewise uninterpretable for similar reasons.

Schoenhuber and Gentilini (1989) studied the influence of exposure to styrene on neuropsychological functions (memory and attention). The influence of exposure to styrene on neuropsychological functions (memory and attention). Schoenhuber and Gentilini (1989) studied. The study involved 55 Italian workers (38 males; 17 females). The subjects were grouped according to their urinary styrene metabolites (MA + PGA); no styrene environmental measurements were taken. All subjects were first evaluated on a Friday after 4 work d, and then again on Monday following a weekend break with no known styrene exposures. The mean urinary metabolite concentrations were greater than 700 mg/l for 14 of the subjects on Friday. Comparing the mean test scores of these individuals with the 41 subjects with low urinary metabolite concentrations showed a markedly poorer performance for the group high in metabolite concentrations on Friday. No differences were found between the two groups when the tests were repeated on Monday. The authors pointed out that long-lasting effects of styrene can be excluded, consistent with the field studies by Cherry and Gautrin (1990). Moreover, the authors noted that results of behavioral performance test batteries must be interpreted with caution when assessing occupational exposures since the abnormalities seen after exposure to toxic agents are similar to those occurring in other CNS disorders as well as with chronic alcohol and drug abuse.

7.10.3 Ototoxicity

Studies by Pryor *et al* (1987) showed evidence of hearing loss in rats subjected to an extreme styrene exposure regimen (800 ppm, 14 h/d, 7 d/wk, for 3 wk). Both behavioural auditory responses as well as brainstem evoked responses were diminished at multiple frequencies. Subsequent studies by Albee *et al* (1993) showed hearing loss in rats exposed to 800 ppm, 6 h/d, for 13-wk. The auditory dysfunction occurred at mid and high frequencies (16 and 30 kHz, respectively), but not at frequencies lower than 8 kHz. Neuropathological examinations revealed hair-cell lesions in the cochlea's of these animals. There were no adverse effects on hearing of animals exposed to 50 ppm or 200 ppm styrene, 6 h/d, for 13-wk. This study clearly demonstrates that very severe styrene exposure regimens are required to cause auditory dysfunction in experimental animals while more moderate exposures had no adverse effects on hearing.

Muijser *et al* (1988) conducted a cross-sectional human study to compare auditory thresholds across a wide range of frequencies in styrene-exposed and non-exposed workers. Styrene exposure concentrations were measured on three consecutive days, although it is unclear whether measurements were taken for all workers or just a sub-sample of them. The styrene-exposed individuals were divided into two subgroups, consisting of those directly and those indirectly exposed. The mean exposure concentrations for the indirectly and directly exposed groups were reported to be 14 ppm and 52 ppm, respectively. The study included 59 male styrene-exposed workers and a control group of 94 male workers involved in the production of photographic film. Hearing thresholds were measured in an audiometric booth placed in a mobile test room. There were no significant differences between control and high exposure groups, although there was an equivocal difference between the directly exposed and indirectly exposed groups at 8 kHz. Duration of exposure was not considered. The study did not demonstrate an association between styrene exposure and hearing loss in man. No information was given on factors that could influence hearing such as ear infections, use of antibiotics and the like.

Dr. Sass-Kortsak (1993) measured hearing acuity in 299 subjects, using pure tone screening audiometer, at the beginning and end of a single workshift. On the same day a personal time-weighted average exposure to styrene was measured. In addition information was obtained about previous work history including exposures to noise and chemicals, use of protective equipment for noise or solvents, personal and family history of hearing problems and smoking. No important relationship between styrene exposure and changes in hearing acuity was found over the course of the workshift. Thus styrene does not have an acute effect on hearing. Similarly no conclusive evidence was found for a chronic induced effect when noise was taken into account. As expected age and noise exposure were two important determinants in hearing loss. No conclusive evidence was found for a relationship between smoking, recreational noise, solvent exposures and hearing loss.

Human data show no evidence for a causal relationship between acute or chronic exposure to styrene and prevalence of hearing loss.

7.10.4 Colour Vision

A relatively new and potentially important physiologically endpoint is the described relationship between styrene exposure and impairment of colour vision. In perhaps the first report on this Gobba *et al* (1991) investigated colour vision, in 73 exposed and 57 controls (subject varied in age 16 to 64 and both sexes selected from 7 small fibreglass-reinforced plastics factories). He used the Lanthony 15 Hue Desaturated panel "specifically suited to identify mild to moderate acquired dyschromatopsia" The exposed and controls were matched for alcohol and cigarette consumption. There would remained still to be appreciable differences in the age and sex distribution of the groups. As a whole the styrene-exposed workers showed a significant impairment in colour vision as compared with the control population. When the exposed group was divided into low (< 50 ppm) and high (50 ppm to approximately 130 ppm) exposures the high exposure group was significantly worse than the low. In a follow-up study Gobba and Cavalleri (1993) investigated the reversibility of the effect by examining a group of 39 workers before and after one month vacation. No tendency to restoration was seen.

In an other study Mergler *et al* (1992) reported a high prevalence of acquired colour vision loss among workers exposed to high levels of styrene. Using a similar testing procedure as described by Gobba *et al* (1991) the authors assessed colour vision loss qualitatively. The participants were placed into a high (urinary mandelic acid level > 0.60 nmol/mmol creatinine i.e. corresponding to approx. 50 ppm styrene in air) and a low (urinary mandelic acid level < 0.60 nmol/mmol creatinine) exposed group. The distribution of acquired a loss was significantly different between the two groups; 52% in the high exposure group had acquired dyschromatopsia and 26% in the low exposure group. In addition to these studies Fallals *et al* (1991) also reported acquired colour vision loss associated with exposure to styrene.

All studies have a number of problems including lack of historical exposure data or work histories, lack of detail on the testing regimen and some doubts about the analyses of the test data. Perhaps the most important concern relates to the design of the studies which does not take into account the initial pre-exposure health status of the participants. The differences may have been present already before exposure began. It will be necessary to conduct a longitudinal study measuring changes from baseline values and addressing causal relationships. Similarly the testing regimen needs to be refined to provide a clear definition of what constitutes an effect on colour vision acuity. Currently the styrene industry is exploring ways of conducting further work into this complex problem.

7.10.5 Neurotoxicity Summary

In summary, there is good evidence that styrene can cause feelings of fatigue and drowsiness in humans at exposure concentrations well above 100 ppm. There is some evidence that feelings of fatigue may occur at exposure concentrations around 100 ppm. Pre-narcotic symptoms such as headache, dizziness, and nausea also occur at exposure concentrations above 100 ppm. No other symptoms have been convincingly demonstrated with exposures at or below 100 ppm. Slowing of reaction time in the morning has been observed in several studies where exposures were estimated to be above 50 ppm. This early morning slowing of reaction time seems to occur primarily in workers who have not cleared styrene and its

metabolites from previous exposures. The reaction times of these workers generally tend to improve during the course of the work day, and there is very little evidence that slowing occurs during acute exposures. No other behavioural tests have shown styrene effects at or below 100 ppm. Concentrations over 100 ppm may result in at least transient changes in neurological tests; subtle changes may become apparent at around 100 ppm. There is evidence that at least some individuals have EEG changes at 100 ppm. The EEG changes have not been associated with any specific symptom, and their significance to the health and safety of workers is unknown.

Changes in visual evoked response have been identified rather consistently at exposure concentrations around 100 ppm. No gross clinical effects have been observed in assessments of the peripheral nervous system. There is no convincing evidence of neurotoxic effects due to cumulative exposure, and there is no evidence of any permanent or irreversible effects that persist after exposures have been discontinued and styrene and its metabolites have been cleared.

There is no evidence of toxicity in humans exposed to styrene while the information of an adverse effect on colour vision is limited.

In a critical review of the neuroepidemiology data (Rebert and Hall, 1994) the authors concluded that many of the so called neurological effects seen at lower exposures, i.e. 20 to 50 ppm, were in fact "false positive" outcomes due to statistical error, action of some factor other than styrene and/or misinterpretation of the data.

7.11 IMMUNOTOXICITY

There is very little information on the immunological effects of styrene. The majority of the haematological studies have failed to provide any convincing evidence for styrene having an adverse effect on leucocytes and in a study on the concentrations of serum gamma globulin among workers exposed to different concentrations of styrene no adverse effects were noted (Chmielewski *et al* 1973). The World Health Organization (WHO, 1983) reviewed a Russian study (Sinitsjki, 1969) in which the phagocytic activity of leucocytes from rabbits fed styrene (250 mg/kg for 58 d, 5 mg/kg for 216 d or 0.5 mg/kg for 202 d) was examined. Although no statistical analysis was provided the authors reported a dose-response relationship for both the severity of effect and the time of onset.

8. GAPS IN KNOWLEDGE

The most important need is a clarification the data from existing data studies on animal carcinogenicity. Results are confused by marginal effects and lack in the adequate description of dose response relationship. To clarify the situation the styrene industry in the USA is sponsoring 2 year inhalation studies in the rat and the mouse. Results of the studies, which are expected 1996/1997. These data together with the comprehensive epidemiological information (see recent reports from Kogevinas *et al*, 1994 and Wong *et al*, 1994) should determine whether there is a causal relationship between styrene exposure and an increase in the risk of cancer.

As described previously there is some evidence for the presence of SO-DNA adducts in human white blood cells based on comigration of adduct spots with standards e.g. Vodick *et al* 1993. Further studies are clearly required to confirm such preliminary findings and the European styrene industry is supporting research which will examine if adducts can be detected in humans using ^{32}P-postlabelling techniques.

The report from the US Department of Health and Human Services (1992) identified a number of areas where there was a need for data including basic information on adverse effects of dermal exposure, toxicokinetics, genotoxicity, cancer effects, epidemiology, developmental and immunotoxicity. A number of these have already been discussed i.e. genotoxicity and cancer while data on epidemiology and toxicokinetics have been developed very recently. The other data gaps identified in the US report are not regarded as being critical for establishing an occupational exposure guideline.

9. GROUPS AT EXTRA RISK

Styrene, at high exposure levels, is an irritant and can affect the central nervous system. Workers with the highest potential exposures i.e. in the reinforced plastics industry, are at the greatest risk of suffering such effects. Persons with pre-existing respiratory or neurological problems will be at greater risk for this irritant action and central nervous system depressant effects.

10. EXISTING OCCUPATIONAL EXPOSURE LIMITS

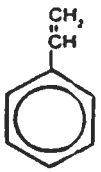
The maximum concentrations of styrene at the workplace for a number of countries, are summarised below:

Country	8 h TWA (ppm)	STEL (ppm)
Austria	20	40 (30 mins)
Belgium	50	100 (15 mins)
Denmark	25 *	
Finland	20	100 (15 mins)
France	50	
Germany	20	40 (30 mins)
Italy	50	100 (15 mins)
Luxembourg	20	40 (30 mins)
Netherlands	50	
Norway	25	37.5 (15 mins)
Spain	50	100 (15 mins)
Sweden	20 **	50 (15 mins)
Switzerland	50	100 (4x10 mins)
U.K.	100 ***	250 (10 mins)
U.S.	50	100 (15 mins)

* This will be a ceiling limit when Denmark publishes next list of exposure limits
** New installations must meet a limit of 10 ppm
*** Maximum Exposure Limit, duty to reduce as low as possible

11. SUMMARY EVALUATION AND RECOMMENDATION FOR A SCIENTIFIC BASED OCCUPATIONAL EXPOSURE LIMIT

11.1 SUBSTANCE IDENTIFICATION

Common name:	Styrene
CAS registry No:	100-42-5
EEC labelling:	R: 10-20-36/38 S: 23
IUPAC name:	Ethenylbenzene
EINECS No:	2 028 515
Synonyms and Styrene trade names:	Vinylbenzene Ethenylbenzene Phenylethylene Phenylethene Cinnamene
Chemical group:	Unsaturated hydrocarbon
Formula:	C_8H_8
Structure:	
Molecular mass:	104.15
Purity of technical product	up to 99.9%

11.2 OCCURRENCE AND USE

11.2.1 Chemical and Physical Properties

Styrene (ethenylbenzene) is a colourless viscous liquid with a pungent odour - detection threshold being described at about 0.02 ppm (70 µg /m^3). It has a vapour pressure of 0.8666 kPa; is soluble in a variety of organic solvents but only slightly so in water. Styrene will polymerise readily in the presence of oxygen and oxidises on exposure to light and air. The commercial product is therefore stored in an inert atmosphere or, more usually, with an added inhibitor such as 0.001% tertiary butylcatechol.

Conversion factors for styrene are:
1 ppm in air = 4.2 mg/m^3
1mg/m^3 in air = 0.24 ppm

11.2.2 Occurrence and Use

Styrene is an important chemical used in the production of polymers, copolymers and reinforced plastics. The presence of styrene in ambient air is probably exclusively of human origin being associated with the chemical industry and perhaps more importantly car exhaust emissions. While it has been suggested that industrial sources are the most likely source of exposure in a study styrene emissions in the Netherlands 1,041 tons was attributable to stationary sources while 1,400 tons was associated with mobile sources. Other sources of styrene are minor importance, they include cigarette smoke and other combustion/pyrolysis processes.

In addition to exposure via air styrene is known to occur naturally in a variety of food products including fruits, vegetables, dairy products, fish, meat, alcoholic and non-alcoholic beverages, nuts and various miscellaneous food groups. In general such products contain only low amounts (i.e. ~ 0.1 µg/kg) although higher levels have been found in variety of food types including beer (10 - 200 µg/kg), turkey sausage (100 µg/kg) and roasted coffee (20 - 300 µg/kg) and in cinnamon in particular (up to 39 mg/kg).

11.2.3 Exposure Levels at the Workplace

The level of occupational styrene exposure is dependent on the type of operation. In plants producing styrene exposures are low, probably not exceeding 5 ppm. Similarly in the manufacture and processing of styrene based polymers exposures are unlikely to exceed 5 ppm and in the majority of cases are usually well below 1 ppm. The only industry with significant exposures is the reinforced plastics industry where styrene has a dual role as a solvent and reactant for producing polyester resins. Because of the nature of this industry where resin is applied to strand mats by hand (paint brush/roller) and spray up formulations, exposures can be high with short term exposures exceeding 100 ppm. Since the industry includes many small firms often employing less than 10 people owners often find it difficult, if not impossible for economical reasons, to implement the appropriate engineering

safeguards. While the reinforced plastics industry consumes only a small proportion of total styrene production (i.e. about 4%) it has by far the highest occupational exposures. A number of initiatives have been taken by both the styrene and reinforced plastics industry to help resolve this problem. Research by the chemical industry has failed to find a substitute for styrene providing the same performance. The reinforced plastics trade association in Europe has with the help of the styrene industry introduced, in 1993, a code of practice aimed at ensuring companies to operate at or below national guidelines.

11.2.4 Exposure Levels in the Environment

Estimated daily intake by the general population of styrene due to its presence in ambient air have been calculated at 23 µg/d and lower. Styrene intake will be higher for smokers possibly as high as about 300 µg/d. EPA and WHO estimated safe concentrations in air at 1 mg/m^3 and 0.8 mg/m^3 respectively. This equates to a daily intake of about 14,000 µg/d. The actual daily intake does not present a health problem.

11.3 HEALTH SIGNIFICANCE

The health effects of styrene have been extensively investigated during the past 40 years.

Acute exposure to styrene is lethal only at high concentrations (inhalation LC_{50}s range from 2,700 to 6,000 ppm; oral LD_{50}s from 316 to 5,000 mg/kg). Styrene is no more than moderately irritating to skin on a single exposure, but repeated exposures can cause defatting and dermatitis. Eye irritation is produced by contact with liquid styrene or by prolonged exposure to styrene vapour concentrations of approximately 100 ppm. Similarly, styrene vapours produce nasal irritation. A styrene vapour concentration of 160 ppm caused a 50% decrease in the respiratory rate in mice, an indication of sensory irritation.

Because of the irritant nature of styrene vapour this must be included in any criteria for establishing the occupational exposure limit.

Styrene is well absorbed by all routes of exposure and once absorbed is distributed throughout the body, especially concentrating in the fat. Styrene is cleared relatively rapidly from the body, and at low doses there is no tendency towards bioaccumulation in any organ or tissue. Studies examining the potential for specific organic toxicities in humans were negative for the haematopoietic system, immune system, kidney, urinary tract, gastrointestinal tract, liver, cardiovacscular and respiratory systems and the endocrine organs (WHO, 1983). Slight effects on the respiratory tract have been noted in some studies although the major responses have been noted on the central nervous system.

High styrene exposure concentrations can cause acute, transient effects on the central nervous system. Pre-narcotic symptoms (headache, dizziness, nausea) and feelings such as fatigue and tiredness occur at exposure concentrations of 100 ppm or greater. Exposure to 100 ppm or greater may result in mild sensory impairment (5-10%) in the peripheral nerves, measured by nerve conduction velocity or sensory amplitude. Some reduction may be observed already between 50 and 100 ppm. There is no evidence of any permanent or

irreversible effects that persist after exposures have been discontinued and styrene and its metabolites have been cleared. Based on the available data the effect of styrene on the CNS is an important factor in establishing an occupational exposure limit.

Styrene is metabolised mostly through epoxidation of the vinyl side chain, forming styrene-7,8-epoxide (SO). Once formed, SO is either conjugated with glutathione or converted to styrene glycol, which is further metabolised to mandelic acid, phenylglyoxylic acid, benzoic acid, and/or hippuric acid which are excreted in the urine. Formation and excretion of styrene metabolites is species specific and influenced by many environmental and lifestyle factors; therefore, measurement of urinary metabolites gives an unreliable estimation of styrene exposure. Recent studies show that mice have the greatest capacity and humans the least to form SO from styrene. In addition, human liver is more effective at hydrolysing low levels of SO formed from styrene. Consequently, at any given styrene exposure concentration, SO levels in humans will be lower than in rodents.

Studies indicate that styrene is not a teratogen. Some studies report embryotoxic or foetotoxic effects, but only at doses toxic to the parents. Initial human studies suggested an association between styrene exposure, congenital CNS malformation and spontaneous abortion. These assertions have been disproved by the same authors in more complete follow-up studies. Overall, there is no evidence that styrene exerts specific developmental or reproductive toxicity.

Scott and Preston (1994) reported that 18 of 52 cytogenetic studies (chromosomal aberrations, micronuclei, sister chromatid exchanges) on peripheral blood lymphocytes of workers in industries in which there is exposure to styrene have reported significant increases in chromosome damage compared with non-exposed controls. The remaining investigations reported negative results. The data suggesting a positive association of clastogenic effects with styrene exposure are however very far from demonstrating convincingly that styrene produces chromosomal damage in exposed individuals. Reason for this include:

(i) many of the studies are based on extremely small numbers of cases;

(ii) the only two reports involving relatively large numbers have shown no association between styrene exposure and clastogenic effects e.g. the most recent data from an extensive EEC supported biomonitoring program showed that styrene exposure concentrations in the range of 5 to 182 ppm did not result in chromosome damage;

(iii) a number of studies report relatively high levels of clastogenic effects which are implausibly high bearing in mind the rest of the published data;

(iv) notwithstanding the large number of studies there is no quantitative relationship between styrene exposure and chromosomal damage; the lack of a dose-effect relationship in such a large group of data is a major confounder for accepting causality between styrene exposure and clastogenic effects.

Support for the proposition that styrene is not responsible for the clastogenic effects reported in workers can be found in the *in vivo* animal data. An examination of the results show that studies in which exposures have been much higher than those encountered in the workplace the results have been overwhelmingly negative. A recent study conducted under the auspices of the US-EPA (Kligerman *et al*, 1992 and 1993) and CIIT (Preston 1993) showed that mice and rats exposed to styrene concentrations up to 500 ppm for two wk and rats exposed to 1,000 ppm for 4 wk showed no evidence of an increase in micronuclei or chromosomal aberrations.

It is well possible that styrene exposure is not responsible for the observed chromosomal changes. Other factors which could be responsible for the results including co-exposure to other chemicals, cigarette smoking, life style etc; confounding factors that could not be excluded in the studies reported (Scott, 1993).

Styrene is not a direct-acting mutagen in *in vitro* bacterial and eukaryotic cell assay systems. With activation, styrene is either non-mutagenic or very weakly mutagenic. Macromolecular binding studies have shown that styrene and styrene oxide have very low potential to react with haemoglobin or DNA.

Because of the many inconsistencies and imponderables associated with the available mutagenic and cytogenetic data it is probably inappropriate to use such information to reach a health based decision on a suitable occupational exposure limit.

The available animal and human data do not indicate that styrene is a carcinogen and it should not be classified as such. A total of nine long-term bioassays have been conducted on styrene, and two additional studies have been performed using a mixture of styrene and β-nitrostyrene. Some of these studies have specific deficiencies and limitations that preclude definitive conclusions. The available data provide no evidence of a carcinogenic response related to styrene exposure. Additional long-term animal studies are currently in progress.

Although there have been some epidemiological studies which suggest there may be an association between styrene exposure and an increased risk of leukaemia and lymphoma the evidence is generally weak. The majority of the studies show no evidence for a causal relationship between styrene exposure and leukaemia and any other form of cancer. The combined weight of evidence of these epidemiological studies, including nearly 50,000 workers in the time period 1940 to 1986, argues against a carcinogenic role for styrene. This conclusion is supported by a study conducted by the International Agency for Research on Cancer (Kogevinas *et al*, 1993) involving over 40,000 workers from the European reinforced plastics industry and an update of the American reinforced plastics industry which included approximately 16,000 workers (Wong, 1994). While the body of evidence from both of these large studies is quite complex the overall result is not supporting the causal link between styrene and cancer, including the occurrence of tumours of the lymphatic and haematopoietic tissues.

11.4 RECOMMENDATION

The major concern with styrene at the workplace, especially in industries where exposures may be quite high, is the possibility of neurotoxic effects. Styrene like many other solvents, volatile anaesthetics and drugs can at certain concentrations produce acute changes in consciousness with subsequent alterations of feelings, cognition and psychomotor function. It is therefore reasonable to use such data to establish an industrial hygiene standard.

At similar concentrations to those producing subjective evidence of irritation (i.e. 100 ppm and above) styrene is reported to cause a range of CNS effects including dizziness, light-headedness, headache and drowsiness with effects getting progressively worse with increasing exposure concentrations. A major debate revolves around what constitutes the NOAEL. Using published data on neurotoxicological effects and exposure information based on creatinine-normalised urinary metabolites Foureman and Jarabek (1993) estimated a NOAEL for humans (using lower confidence limit of the mean) of 94 mg/m3 (22 ppm). However in a detailed review of the neuroepidemiology of styrene Rebert and Hall (1994) concluded that, in general, results often interpreted as showing that styrene is neurotoxic did in fact reflect false positive outcomes due mainly to statistical error, confounding variables and mis- or over-interpretation of data. Although the studies are not consistent, a value of 100 ppm judged to be perhaps the lowest effect level with the qualification that the effects are however transient and small. This supports an industrial hygiene level of 50 ppm with a short term exposure limit (15 minutes) of 100 ppm. Using quantitative skin penetration data (0.06 mg/cm^2/h) it can be calculated (using the procedure outlined in ECETOC DOC. No 31, 1994) that no skin notation is required.

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