EUROPEAN UNION RISK ASSESSMENT REPORT

Styrene

CAS No: 100-42-5

EINECS No 202-851-5

Draft for publication, June 2008

United Kingdom

This document has been prepared by the UK rapporteur on behalf of the European Union.

Foreword

This risk of the priority substance covered by this Draft Risk Assessment Report is carried out in accordance with Council Regulations (EEC) 793/93[1] on the evaluation and control of the risks "existing " substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and if necessary, recommending a strategy to limit the risks of exposure to the substance.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in the Commission Regulation (EC) 1488/94[2] which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented to the Competent Group of Member State experts for endorsement. Observers from Industry, Consumer Organisations, Trade Unions, Environmental Organisations and certain International Organisations are also invited to attend the meetings. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Eco-toxicity and the Environment (SCTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

This Draft Risk Assessment Report is currently under discussion in the Competent Group of Member State experts with the aim of reaching consensus. During the course of these discussions, the scientific interpretation of the underlying scientific information may change, more information may be included and even the conclusions reached in this draft may change. The Competent Group of Member State experts seek as wide a distribution of these drafts as possible, in order to assure as complete and accurate an information basis as possible. The information contained in this Draft Risk Assessment Report does not, therefore, necessarily provide a sufficient basis for decision making regarding the hazards, exposures of the risks associated with the priority substance under consideration herein.

This Draft Risk Assessment Report is the responsibility of the Member State rapporteur. In order to avoid possible misinterpretations or misuse of the findings in this draft, anyone wishing to cite or quote this report is advised contact the Member State rapporteur beforehand.

Contact Details of the Rapporteur(s)

Rapporteur: United Kingdom

Contact (human health): Health & Safety Executive
Chemical Assessment Schemes Unit
Redgrave Court, Merton Road
Bootle, Merseyside L20 7HS

e-mail: ukesrhh@hse.gsi.gov.uk

Tel: +44 151 951 3791
Fax: +44 151 951 4889

CONTENTS

OVERALL RESULTS OF THE RISK ASSESSMENT

CAS Number: 100-42-5

EINECS Number: 202-851-5

IUPAC Name: Styrene

Environment

Conclusion (ii) **There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.**

Conclusion (ii) applies to all scenarios.

Human health

Human health (toxicity)

Workers

Conclusion (iii) **There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.**

The MOSs (Margins of Safety) for acute CNS (Central Nervous System) depression, skin, eye and respiratory tract irritation, effects on the ear and colour vision discrimination following repeated exposure and developmental toxicity for GRP (glass-reinforced plastic) manufacture are unacceptably low, and therefore conclusion (iii) applies. Conclusion (iii) also applies to production of UP (unsaturated polyester)-resin in relation to effects on the ear following repeated exposure and developmental toxicity and to production of SBR (styrene-butadiene resin) and SB (styrene-butadiene) latex in relation to developmental toxicity.

Conclusion (ii) **There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.**

For all remaining scenarios (manufacture of the monomer and production of polystyrene), the MOSs for acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear and colour vision discrimination following repeated exposure and developmental toxicity are considered to be sufficient, and therefore conclusion (ii) applies. Comclusion (ii) also applies to all scenarios in relation to sensitisation, mutagenicity, carcinogenicity and effects on fertility.

Consumers

Conclusion (iii) **There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.**

Conclusion (iii) applies to exposures arising from boat building in relation to acute CNS depression, eye and respiratory tract irritation, effects on the ear and on colour vision following repeated exposure and developmental toxicity. Conclusion (iii) also applies to the use of styrene containing liquid resins in relation to eye and respiratory tract irritation, effects on the ear and on colour vision following repeated exposure and developmental toxicity, and to the use of styrene-based paste in relation to developmental toxicity.

Conclusion (ii) **There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.**

Conclusion (ii) applies to long-term low level exposures arising as a result of emissions from polymeric building materials, including carpets, from food sources (mainly as a consequence of food packaging) and from chewing gum in relation to acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear, colour vision discrimination and hepatotoxicity following repeated exposure and developmental toxicity. Conclusion (ii) also applies to exposures arising as a result of emissions from newly laid carpets in relation to acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear following repeated exposure and developmental toxicity. Conclusion (ii) applies to the use of styrene containing liquid resins in relation to acute CNS depression, skin irritation, to the use of styrene-based paste in relation to acute CNS depression, skin, eye and respiratory tract irritation and effects on the ear following repeated exposure and to exposures arising from boat building in relation to skinirritation. Conclusion (ii) applies for all scenarios in relation to sensitisation, mutagenicity, carcinogenicity and effects on fertility.

Humans exposed via the environment

Conclusion (ii) **There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.**

Conclusion (ii) applies to both regional and local exposures in relation to all endpoints.

Combined exposure

Conclusion (ii) **There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.**

Conclusion (ii) applies to a consumer exposed to styrene from polymeric products, from food and from chewing gum, who is also exposed via the environment. This conclusion applies in relation to all endpoints.

Human health (physico-chemical properties)

Conclusion (ii) **There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.**

Conclusion (ii) applies.

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS no: 100-42-5

EINECS no: 202-851-5

IUPAC name: Benzene, ethenyl

Molecular formula: C_8H_8

Structural formula: $C_6H_5CH=CH_2$

Molecular weight: 104.15

Styrene is a liquid at room temperature. The most common synonyms are cinnamene, ethenyl benzene, phenylethene, phenylethylene and vinylbenzene, together with the equivalent names in other languages. Styrene polymerises at room temperature in the presence of oxygen and oxidises in the presence of light and air. It is slightly soluble in water, but soluble in most organic solvents.

1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Purity

The suppliers state that the purity varies from 99.7% to greater than 99.9% w/w.

The impurities (where stated in IUCLID) as % w/w comprise some or all of the following:

Ethylbenzene	<0.1%
Isopropylbenzene (cumene)	<0.1%
2-Phenylpropene	<0.1%
Water	<0.025%
Phenyl acetate	<0.02%
p-Xylene	<0.06%
m-Xylene	<0.001%

The impurities vary with the plant and production method.

1.2.2 Additives

The only stated additive was 4-tert-butylpyrocatechol (4-tert-butylbenzene-1, 2-diol), which is added as a polymerisation inhibitor at <0.006 – 0.01% w/w.

1.3 PHYSICOCHEMICAL PROPERTIES

Data on the physicochemical properties of styrene have been obtained from the IUCLID entries, from handbooks and from a number of other sources including a monograph on styrene. Neither the IUCLID data (which frequently quote handbooks) nor the handbooks

give the conditions under which the tests were carried out, particularly the purity of the styrene used. Furthermore, while the literature may imply that there are a large number of measurements within a narrow range, suggesting a degree of consistency between independent measurements, the lack of reference to original papers means that it is possible that all of these are derived from one source value. Consequently it is hard to assess the quality of some of the data available.

The styrene monograph (Boundy and Boyer, 1952) provides data on highly purified material (99.94 – 99.95% pure), which is of similar order of purity to that sold commercially (99.7 - >99.9%). The monograph also provides details of test conditions used and reference to other original papers.

1.3.1 Physical state (at ntp)

Commercially-produced styrene is a colourless to slightly yellow volatile liquid with a sweet and pungent odour. The odour threshold is reported to be 0.15ppm (BASF, 1989).

Styrene polymerises on exposure to light and air, but it can also oxidise to form certain aldehydes and ketones giving a sharp, penetrating, disagreeable odour (Boundy and Boyer, 1952).

1.3.2 Melting Point

The melting point of styrene has been measured at –30.6, -30.7 and -31°C. The first value was presented in the consolidated IUCLID data set (BASF, 1990) and is the value quoted in the Merck Index (11th Edition, 1989), by Mackay *et al* (1993), and by Kirk-Othmer (4th Edition, 1991). The latter two figures were presented in a second IUCLID entry and the CRC Handbook (75th Edition, 1994) respectively.

Boundy and Boyer (1952) give a value of -30.63°C for high purity (99.4 – 99.5%) styrene, together with original literature references and experimental details.

1.3.3 Boiling Point

The boiling point of styrene has been placed between 145 and 146^{0}C. A value of 145.2^0 C is presented in the consolidated IUCLID date set (BASF, 1990). A range of 145 - 146^0 C is presented in the Merck Index (11th Edition, 1989) and the CRC Handbook (75th Edition, 1994), and by Mackay *et al* (1993).

The value of 145.2°C is also presented in Boundy and Boyer (1952) as the boiling point for high purity (99.4 – 99.5%) material.

1.3.4 Relative Density

The relative density of styrene has been measured at 0.901 – 0.906 at 20°C. The latter value is presented in the consolidated IUCLID data set (BASF, 1990), in the Merck Index (11th Edition, 1989), the CRC Handbook (75th Edition, 1994) and Mackay *et al* (1993). A value of 0.907 at 19.9°C is presented in Beilstein, while a value of 0.9014 at 25°C is presented by Chaiyavech *et al* (1959).

A figure of 0.906 at 20°C is represented in Boundy and Boyer (1952) for high purity material (99.4 – 99.5%). The original literature references describe the experimental methods used.

1.3.5 Vapour Pressure

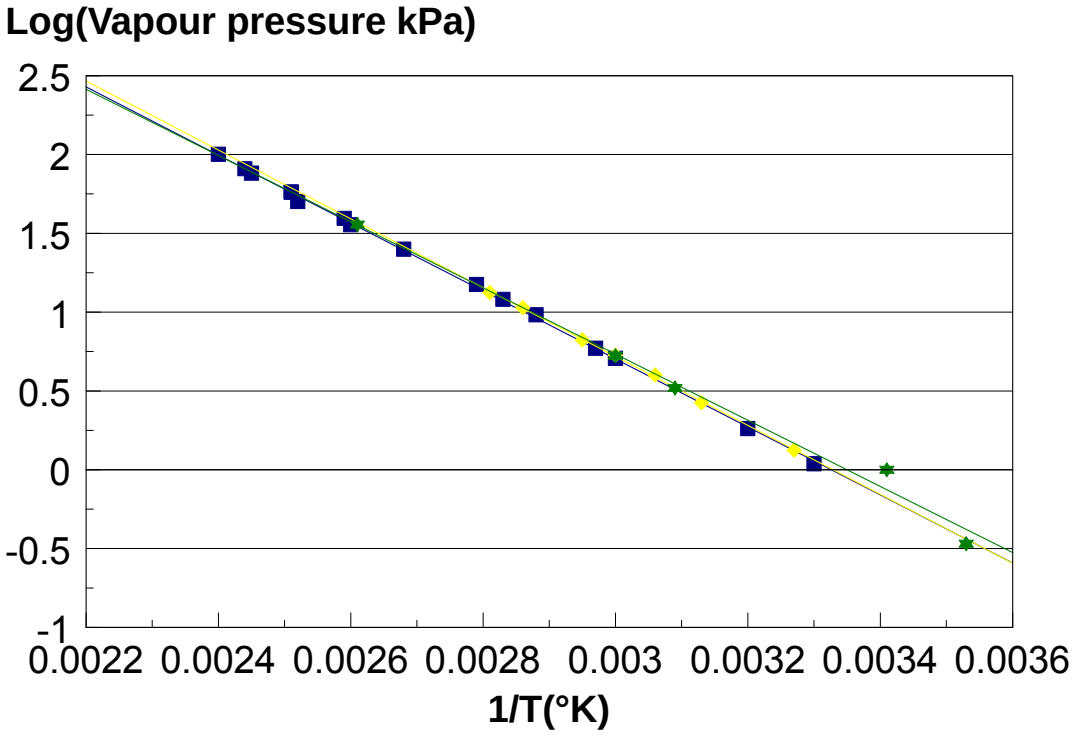
A number of individual values for the vapour pressure of styrene have been presented both in IUCLID and from other sources: 3.4 hPa (0.34 kPa) at 10°C (Shell, unpublished); 6 - 10 hPa (0.6 – 1.0 kPa) at 20°C (BASF, 1990); 10 hPa (1 kPa) at 20° (Elf Safety Data Sheet, quoted in IUCLID); 6.67 hPa (0.667 kPa) at 20°C, 12.66 hPa (1.266 kPa) at 30°C (Verscheuren, 1983); 33 hPa (3.3kPa) at 50°C (BASF, 1990); 53 hPa (5.3 kPa) at 60°C (unreferenced); 360 hPa (36 kPa) at 110°C (Shell, unpublished).

In addition to these, two sets of original data have been obtained and these are presented in **Table 1.1** and plotted in **Figure 1.1** (Dreyer *et al,* 1954; Chaiyavech *et al,* 1959).

Table 1.1 Vapour pressure data

Dreyer *et al* (1954)		**Chaiyavech *et al* (1959)**	
Temperature (°C)	**Vapour Pressure (kPa)**	**Temperature (°C)**	**Vapour Pressure (kPa)**
29.92	1.093	32.4	1.333
39.21	1.827	45.6	2.666
60.04	5.106	53.86	4.001
63.23	5.906	60.05	5.333
74.42	9.639	65.45	6.666
80.17	12.066	76.6	10.666
85.53	14.999	82.19	13.31
99.51	25.105		
110.06	35.877		
113.1	39.383		
123.81	50.329		
125.39	57.662		
125.41	57.915		
134.83	76.207		
137.23	81.326		
144.77	100.511		

Figure 1.1 Vapour pressure



The two sets of data are consistent with each other but unfortunately do not cover the range of greatest interest for the environmental assessment, between 10 and 30°C. However, it is possible to extrapolate into this range to predict vapour pressure and to compare the predicted values with those quoted above to check their consistency.

The plot suggest that the critical vapour pressures are approximately 0.6 kPa at 20°C, 1 kPa at 25°C and 1.6 KPa at 30°C, suggesting that the “other information” including IUCLID data is consistent with the two sets of series information.

The value quoted by Verscheuren for 20°C, 6.67 hPa (0.667 kPa), has been used for environmental modelling purposes later in this report.

1.3.6 Solubility

Styrene is slightly soluble in water (300mg/1 at 20°C). This value was presented in the consolidated IUCLID data set (BASF, 1990) and in Verscheuren (1983) and Mackay *et al* (1993). Kirk-Othmer (4th Edition, 1991) presents an additional value of 302 mg/1 at 25°C.

Values of 0.029 g/100 g of water (290 mg/1) at 20°C and 0.034 g/100 g of water (340 mg/1) at 30°C are presented in Boundy and Boyer (1952), referenced to the original literature (Lane, 1946).

The solubilities presented for styrene are consistent with variations in purity, a degree of experimental variability and certain amount of rounding of values. A value of 300 mg/l is used for environmental modelling purposes in this report.

Styrene is miscible in all proportions with organic solvents such as ether, methanol, ethanol acetone, benzene, toluene and carbon tetrachloride.

1.3.7 Partition Coefficient

Three measured values for the octanol-water partition coefficient (as log Kow) are included in the IUCLID data set. These are 2.95 (Hansch and Leo, 1979), 2.96 (BASF, 1987) and 3.16 (Banerjee *et al,* 1980) and 3.05 (Sangster, 1989). A value of 2.89 has been calculated using the SRC program KOWWIN.

The BASF measured value used OECD method 107, described in IUCLID as the shake-flask method. The styrene was of high purity (99.7%); the concentration in the water phase was measured, and the concentration in the octanol phase was estimated by difference. The value from Hansch and Leo (1979) comes from an unpublished analysis, most likely using the shake-flask method. Banerjee *et al* (1980) employed a version of the shake flask method using stainless steel centrifuge tubes and precautions to minimise volatility. They measured the concentration in both phases. The exact purity of the material used in these last two determinations is not known. The variability observed is in line with that expected from the method used. The value proposed by Sangster (1989), 3.05, appears to be the mean of the two values available in the open literature (Hansch and Leo and Banerjee *et al*). This value was recommended by Mackay *et al* (1993) in their review of the data for styrene. Mackay *et al* (1993) also quoted two determinations by HPLC, giving values of 2.76 (Fujisawa and Masuhara 1981) and 2.90 (Wang *et al*, 1986).

In view of the agreement between the shake flask results, the mean log value from these studies has been used in the environmental modelling in this risk assessment (this is equivalent to taking the geometric mean of the actual measurements). This gives a value of 3.02.

1.3.8 Flash Point

Values for the flash point, measured by the closed cup method, are presented as 31.0°C (BASF, 1990) and 34.4°C (NIOSH, 1983, quoted in a second IUCLID entry). Values measured by the open cup method are presented as 31.1 and 34.4°C (Kirk-Othmer, 4th Edition, 1991).

A flash point of 31°C (open cup to ASTM standard) is presented in the styrene monograph (Boundy and Boyer, 1952). This figure is also quoted by the NFPA (1994). The latter quotes the fire point (the temperature at which the substance continues to burn once the source of ignition is removed) as 34°C.

The values presented can be considered as valid, noting that variation may occur depending on the purity of the styrene, the precise definition of “flash point” and the observation characteristics of the particular test method. The flash point of styrene will be considered to be 31°C.

1.3.9 Auto flammability

The auto flammability (auto ignition) point of styrene has been quoted as 490°C in the consolidated IUCLID data set (BASF, 1990). The same value is quoted in the Merck Index (11th Edition, 1989) and Kirk-Othmer (4th Edition, 1991), the National Fire Protection Association (NFPA, 1994) and Boundy and Boyer (1952), the latter referring to a test method according to ASTM guidelines.

The auto ignition point will be considered to be 490°C.

1.3.10 Explosivity

Styrene is not explosive as defined by Directive 67/548/EEC, on the basis of structure and oxygen balance calculations (Annex V to 79/831/EEC). However, the heavy vapour may burn explosively if ignited in an enclosed area (McLellan, 1994).

Flammability limits are quoted as 0.9 (lower) and 6.8 (higher) (NFPA, 1994) and 1.1 to 6.1 (quoted as a percentage in air, McLellan 1994). The latter set of figures is also quoted by Boundy and Boyer (1952). The NFPA data are considered to be the best estimate since the values quoted by Boundy and Boyer have been calculated from vapour pressure studies, rather than measured test data.

Styrene can polymerise explosively in the absence of a stabiliser (McLellan, 1994). Styrene oxidises slowly in the presence of light and air to form peroxides.

1.3.11 Other Physicochemical Properties

The vapour density of styrene is quoted as 3.6 (air=1) (WHO, 1983).

1.3.12 Summary

Where several values for the physicochemical properties of styrene are available, these are generally within a narrow range, consistent with experimental variability.

There is no value for the surface tension but this is not considered to be critical for the risk assessment.

Table 1.2 presents the values for the physicochemical properties of styrene as used in this assessment report.

Table 1.2 Physicochemical properties of styrene

Property	Value
Physical state	Liquid
Boiling Point (at 1 atmosphere)	145 - 146°C
Melting Point	-30.6°C
Vapour Pressure	5 mmHg (667 Pa) at 20°C
Water Solubility	300 mg/1 at 20°C
Octanol- Water Partition Coefficient	3.02 (log value)
Density	0.906 g/cm^3 at 20°C
Vapour Density (air=1)	3.6
Flash point (closed up)	31°C, 34.4°C
Auto flammability	490°C
Conversion Factors	1mg/m^3 = 0.23 ppm; 1 ppm = 4.33 mg/m^3

1.4 CLASSIFICATION

Styrene is classified as a dangerous substance within the meaning of Directive 67/548/EEC and is listed in Annex I of this Directive, being assigned risk and safety phrases:

R10:	Flammable
Xn; R20:	Harmful by Inhalation
Xi; R36/38	Irritating to eyes and skin
S2:	Keep out of reach of children
S23:	Do not breath gas/fumes/vapour/spray.

For human health the following classification and labelling was agreed by the TC C&L at their meeting in September 2007:

R10:	Flammable
Xn; R20:	Harmful by Inhalation
Xi; R36/38/37:	Irritating to eyes, skin and respiratory system
Xn; R48/20	Danger of serious damage to health by prolonged exposure via inhalation

[Repr Cat 2;R61/Repr Cat 3;R63/NC]

No agreement could be reached in relation to developmental toxicity. This endpoint will be finalised in the follow-up period or by further discussion.

There is currently no classification for the environment.

The acute toxicity values for fish, daphnia and algae all lie between 1 and 10 mg/l, which is the range for R51. Styrene is readily biodegradable and so is not expected to persist in the environment. However, styrene has a log Kow value of ~3 so it may accumulate in organisms. An experimental study which indicated that styrene did not accumulate to the levels expected from its Kow value is not considered valid. The available data therefore leave styrene on the borderline for classification. No further testing is required for the risk assessment as the available data are sufficient for this purpose. Rather than propose any testing solely for the purpose of classification, a search of IUCLID for information on related substances has been carried out. The information found for substances such as toluene, xylene and ethylbenzene shows that they do not accumulate to any great degree in aquatic organisms. By analogy with these substances and on the basis of the balance of the information available it is concluded that styrene will not accumulate in aquatic organisms and that R53 is therefore not appropriate. The proposal is that styrene is not classified as dangerous to the environment.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION METHODS

Styrene is produced commercially from crude oil by a sequence of processes.

Steam cracking of naphtha obtained from the refining of crude oil produces ethylene, propylene and a mixture of monocyclic hydrocarbons including benzene. Ethylene and benzene, fractionated from this mixture, are then reacted together in the presence of a catalyst to produce ethylbenzene.

Styrene is manufactured from ethylbenzene by one of two routes (Reinders, 1984 and WHO, 1983). Firstly, it can be manufactured by dehydrogenation:

$$PhC_2H_5 \xrightarrow{\text{catalyst}} PhCH{=}CH_2 + H_2$$

Iron oxide is used as a catalyst, together with zinc and magnesium oxides. Steam is added as a dilution agent and to improve the heat transfer. The reaction is carried out at approximately 700°C and 0.8 bar. The purification of the reaction product is done by vacuum distillation. To prevent the polymerisation of the styrene, the conversion is carried out to only 60%, and there is always a reasonable dilution. The by-product gases formed in this reaction are used as a fuel or they are flared.

Alternatively, styrene may be manufactured by oxidation of ethylbenzene to the hydroperoxide by bubbling air through the liquid reaction mixture. They hydroperoxide is then reacted with propylene to yield propylene oxide and a co-product, methyl phenyl carbinol, again in the liquid phase. The carbinol is dehydrated to styrene over an acid catalyst at about 225°C. This reaction is shown below.

$$PhCH_2CH_3 + O_2 \longrightarrow PhCH(CH_3)OOH$$

Ethylbenzene → ethylbenzene hydroperoxide

$$PhCH(CH_3)OOH + CH_3CH{=}CH_2 \longrightarrow PhCHOHCH_3 + CH_3HCH_2O$$

propylene → methyl phenyl carbinol + propylene oxide

$$PhCHOHCH_3 \longrightarrow PhCH{=}CH_2 + H_2O$$

Styrene

2.2 Production Volumes

Approximate world production of styrene in 1995 is given as 16.5 million tonnes (Kirk-Othmer, 4th Edition, 1997). From information in IUCLID the range of production in EU countries is 2.22 to 4.91 million tonnes per year and the range of import tonnages is 30,000 to 150,000 tonnes, with nine companies producing or importing styrene in quantities of over 1000 tonnes per annum.

A CEFIC report gives a figure for the production and use of styrene in Western Europe (including some countries not within the EC) as 3,743,000 tonnes in 1993. This is a more accurate estimate than simply adding the maximum capacities and so will be used throughout the report.

2.3 Uses

Styrene is processed in closed systems as an intermediate in the chemical industry. It is the monomer for polystyrene (general purpose, GP-PS; high impact, HI; and expanded EPS) and copolymer systems (acrylonitrile-butadiene-styrene, ABS; styrene-acrylonitrile, SAN; methyl methacrylate-butadiene-styrene, MBS, and others) and in the production of styrene-butadiene rubber (SBR) and related lattices (SB Latex for example). It is also used as a component of unsaturated polyester (UPE) resins. More details of the uses of styrene are given in the sections below (information taken from: Ashford (1994), Buchanan (1989), Heaton (1986), Kirk-Othmer (4th Edition, 1997) and Reigel (1974). **Table 2.1** presents a brief summary of the main uses of styrene polymers and copolymers, worldwide.

Table 2.1 Uses of Styrene polymers and copolymers worldwide

Styrene products	Industrial and consumer applications
Polystyrene, GP-,HI-	General packaging, furniture, electrical equipment (e.g. audio-visual cassettes) industrial mouldings (e.g. dental, medical)
Polystyrene, EPS	Packaging, thermal insulation of refrigeration equipment and buildings
ABS	Interior and exterior automobile parts, drains, ventilation pipes, air conditioning, hobby equipment, casings etc.
SBR	Tyres, radiator and heater hoses, belts and seals, wire insulation
SB Latex	Paper coatings, carpet backings, floor tile adhesives
UPE resins – glass reinforced	Building panels, marine products, household consumer goods, trucks
UPE resins – non reinforced	Casting resins used for producing liners and seals, in putty and adhesives

The distribution of styrene usage in production of polymers and copolymers in Europe (1986 and 1993) and worldwide is presented in **Table 2.2**

Table 2.2 Usage of Styrene monomer

Use	World 1993[a]		Europe 1993[b]		Europe 1986[c]	
	000 tonnes	%	000 tonnes	%	000 tonnes	%
GP-, HI-PS	8063	56.7	1879	50.2	2000	64
EPS	1519	10.7	696	18.6		
ABS	1559	11	397	10.6	360	11
SAN	271	1.9				
SB Latex	1027	7.2	389	10.6	410	13
SB Rubber	1011	7.1	209	5.6		
UPE Resin	289	2	172	4.6	145	5
MBS	26	0.2				
SB Resins	26	0.2				
Miscellaneous	418	2.9			210	7

Sources a – Miller *et al,* 1994
b – BP personal Communications, derived from 1993 CEFIC figures
c – BUA 1990

Detailed breakdowns of usage are not available, but in the UK there is some indication from the industry that SBR may be more widely produced than ABS, and the quantity of styrene monomer used in the production of UPE resins may be higher as a percentage of total styrene use than for Europe as a whole. Within the UK a small number of companies produce polystyrene, SBR AND UPE resin. However there are hundreds of small and medium sized companies using these products: some 700 companies, for example, use UPE resins in the manufacture of glass reinforced plastic and this number is expected to rise.

As an example of breakdown use, the quantity of styrene polymers used in the UK have been estimated by industrial sector as shown in **Table 2.3** below (Jolly *et al,* 1994)

2.3.1 Polystyrene

General-purpose grade polystyrene (GP-PS) is made by either a continuous mass process or by a suspension process. In the continuous process styrene, sometimes mixed with a non-polymerisable volatile diluent, is passed through two or more reactors with several heat exchange zones and agitators. The reaction mixture, now containing ~85% PS together with residual monomer, is then transferred to a low-pressure high temperature devolatilisation tower where unreacted monomer and the diluent are removed and recycled. The hot polymer is then fed into an extruder where the polymer strands are cooled and cut into pellets.

In the suspension process, styrene is dispersed in water in the presence of 0.01-0.05% suspending agent and a polymerisation initiator (usually a mix of organic peroxides). The reaction mix is heated until polymerisation is substantially complete. The polymer beads are washed, dried and pelletised. GP-PS is easily processed to stable mouldings which have a variety of uses.

Table 2.3 Consumption of styrene polymers by application in the UK

	Annual Consumption	
	Ktonnes	**%**
POLYSTYRENE		
Packaging	59.8	31
Housewares	34.2	18
White goods	31.2	16
Brown goods	25.9	14
Miscellaneous	25.3	13
Building	10.0	5
Transport	3.5	2
TOTAL	190	100
EXPANDED POLYSTYRENE		
Packing	26	65
Building	12.4	31
Agriculture	1.6	4
TOTAL	40	100
ACRYLONITRILE-BUTADIENE-STYRENE COPOLYMER		
Brown Goods	27.6	43
White Goods	32.1	37
Packaging	13.8	18
Miscellaneous	1.5	2
TOTAL	75	100

Notes: "white goods" are domestic appliances such as washing machines, fridges etc
"brown goods" are domestic equipment such as TV's, videos etc

High impact polystyrene (HI) is a less brittle form of PS by copolymerisation of styrene with up to 10% of polybutadiene or styrene- butadiene rubber. It too can be made by continuous or suspension processes; the rubber is dissolved (3-10%) in the styrene monomer prior to the polymerisation. In the suspension process, the styrene solution of the rubber is normally polymerised in mass to a conversion of 20-30% before formation of the suspension. HI-PS is used mainly in packaging, in household/office equipment, refrigerator fittings and linings etc.

Information from producers indicates that the continuous process dominates the production of GP- and HI-PS, accounting for 90-95% of the production of these materials. The suspension process is only used for specialist products where particular properties are required.

A third form of polystyrene is expandable polystyrene, EPS, which is mainly produced by suspension polymerisation of styrene with the addition of a blowing agent such as *n*-pentane. It is used in insulation and protective packaging where it is blown out to become expanded polystyrene.

2.3.2 Styrene Copolymers

A second major use of styrene is in the production of copolymer resins, ABS and SAN. Acrylonitrile/butadiene/styrene (ABS) usually consists of a styrene-acrylonitrile copolymer with a grafted disperse phase of polybutadiene rubber. The polybutadiene (or styrene-butadiene) rubber is first polymerised in water to give a latex. Acrylonitrile and styrene monomers are then added to continue polymerization within the latex.

In addition to the emulsion polymerisation described above, ABS can also be made by suspension and continuous mass processes. The suspension process involves dissolving polybutadiene rubber in the styrene and acrylonitrile monomers and adding a free-radical initiator and chain transfer agents. When a monomer conversion of 25-35% is reached the mixture is transferred to a suspension reactor where it is dispersed in water. Once the required degree of conversion has been reached the product is washed, dewatered and then dried.

The continuous mass process also begins with polybutadiene rubber dissolved in styrene and acrylonitrile monomers together with initiators and modifiers. The reaction begins in a pre-polymerise in which the reaction causes ABS rubber to precipitate. When monomer conversion reaches around 30% the mixture is transferred to the bulk polymeriser and the reaction continues to 50-80% monomer conversion. The unreacted monomers are removed and recycled and the ABS is extruded, cooled in a water bath and pelletised. As this process does not use water as the reaction medium the need for dewatering and drying the product is removed and the amount of waste water reduced.

The composition of ABS can vary widely depending on the required properties of the product. Additions such as methyl styrene or methyl methacrylate are also possible depending on the intended end use. A typical composition of ABS would be: 15-25% acrylonitrile, 5-30% 1,3-butadiene and 50-75% styrene. ABS is used on its own or with a range of other polymers in blends.

Styrene/acrylonitrile resins are made by the controlled addition of styrene monomer to a solution of acrylonitrile. Copolymers with a high acrylonitrile content (70-80%) have low gas permeability and are used for containers and other household items.

Styrene is also used to make a number of other copolymers with butadiene, isoprene, methacrylate and methylstyrene amongst others (styrene butadiene rubbers are dealt with below).

2.3.3 Unsaturated Polyester (UPE) Resins

Styrene is added to unsaturated polyester resins to act as a cross-link agent and reactive diluent in the production of glass reinforced plastic. It also acts as a solvent for the resins. The styrene content of the resins can range from 30-50% depending on the degree of cross-linking required. Low styrene emission resins (done by lowering the styrene content or addition of emission reducing additives) are now being used by many companies in the EU.

There are many methods used to mould UPE resins. These include:

(a) Hand lay-up

(b) Spray lay-up

(c) Resin transfer moulding (RTM)

(d) Sheet and bulk moulding compound (SMC and BMC)

(e) Filament winding

(f) Pultrusion process

The hand and spray lay-up techniques are slowly being replaced with some of these other methods, which are on the whole more enclosed. However, open moulding techniques, such as hand and spray lay-up, still represent about 60% of the EU market for GRP moulding.

2.3.4 Styrene Butadiene Rubbers

Styrene butadiene rubber (SBR) is largely made by the co-polymerization of the two monomers in water. Reigel (1974) suggests 1 part styrene to 3 parts butadiene in 8 parts water (by weight), with the monomers dispersed using an emulsifying agent. Catalyst and modifying agent are added and the reaction allowed to proceed to the required degree. The resulting colloidal aqueous emulsion or latex (SBL) is stripped of unreacted monomer (butadiene removed with a compressor and styrene by vacuum steam distillation) and an antioxidant added. The latex can be used directly for some purposes, or the rubber can be isolated from the latex by coagulation, removal and drying.

SB rubber can also be made by solution polymerisation, with the monomers dissolved in solvent. This allows greater manufacturing flexibility. More recent composition figures suggest a styrene content of 20-35% in latex and 23-25% in rubber, with up to 40% in some grades. SB latex is used in adhesives, paper coatings and foams; SB rubber is used in tyres, insulation and moulded rubber goods amongst others.

Other latices can be produced using styrene and butadiene. High styrene copolymers can have styrene contents of 80-85% and are produced by emulsion polymerisation. They are used as impregnating resins and for shoe soling. The addition of 5% carboxylic acid produces a carboxylated latex XSBR, with a solids content of 50-55%. The styrene content varies from 35-85%. They are used in adhesives and binders, for example in carpet underlay. The use of pyridine gives a latex know as PSBR, with 15% styrene, used in tyre cords and drive belt manufacture. In some statistics, particularly those describing the use of styrene, all these latices appear to be counted in together. Statistics on rubber production, on the other hand, tend to separate out of XSBR latices, but include other such as PSBR in general SB latices.

2.4 Releases From Production And Use Sites

The production and usage of styrene monomer within individual EU member states varies, the major producer and user countries being Germany, France, the United Kingdom, Italy and The Netherlands.

In section 3, estimates of releases from production and use sites will be made. For this purpose the sizes of realistic large sites are required. The largest production sites listed in IUCLID have capacities between 500,000 and 1,000,000 tonnes per year. **Table 2.4** lists the capacities of the largest users of styrene identified in Europe, or in the UK where wider information could not be found. The European production in each area is also given.

2.5 Controls

Styrene is an existing substance with a long history of production and use. A number of effective controls to reduce emissions currently exist, whether simply adopted as part of plant design or added later in response to demands to reduce emissions into the workplace or the environment.

No information has been obtained on current legislative controls of emissions into the environment. Within the workplace, there are existing controls relating to particular industries but these are noted in more detail in the section on occupational exposure.

Occupational exposure limits do vary but they should be looked at in the context of the measurement and enforcement regimes used to ensure that the limits are met. The limits in isolation may be misleading in terms of the levels of exposure and consequently or risk that they imply.

The use of styrene as a monomer in food contact plastics is currently authorised under directive 90/128/EEC without a specific migration limit (although an overall migration limit of 60 mg/kg/food applies).

Table 2.4 Largest user of styrene in Europe

Product	Largest Capacity (tonnes)	Notes	European Production (000 tonnes)	Notes
GP-, HI-PS	125,000	a	1,879	
EPS	60,000	a	696	
ABS	50,000	b	794	d
SAN	50,000	e		
UPE	50,000	b	430	f
SBR	160,000	c	951	g
SB Latex	35,000	c	188	g
XSBR	55,000	c	685	g

Notes a - largest user identified from available information
b - largest user in UK from Chem-Intell (1991)
c - largest European user from IISRP (1994)
d - from styrene usage figure for ABS & SAN assuming average styrene content of 50%
e - assumed to be same as ABS
f - from styrene usage figure assuming average styrene content of 40%
g - European capacities from IISRP (1994)

3 ENVIRONMENT

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure Assessment

4.1.1.1 Occupational Exposure

4.1.1.1.1 General Discussion

Definitions and Limitations

In this document, unless otherwise stated, the term exposure is used to denote personal exposure as measured or otherwise assessed without taking into account the effect of any personal protective equipment (PPE) which might be in use. This definition permits the effect of controls other than PPE to be assessed and avoids the problem of trying to quantify the actual protection provided by PPE in use.

The general discussion sections summarise the important issues arising from the exposure assessments and brings together measured exposure data and predictions from the EASE (Estimation and Assessment of Substance Exposure) model. EASE is a general purpose predictive model for workplace exposure assessments. It is an electronic, knowledge based, expert system which is used where measured exposure data are limited or not available. The model is in widespread use across the European Union for the occupational exposure assessment of new and existing substances.

All models are based upon assumptions. EASE is only intended to give generalised exposure data and works best in an exposure assessment when the relevance of the modelled data can be compared with and evaluated against measured data.

EASE is essentially a series of decision trees. For any substance, the system asks a number of questions about the physical properties of the substance and the circumstances of its use. For most questions, the EASE user is given a multiple-choice list from which to select the most appropriate response. Once all the questions have been answered, the exposure prediction is determined absolutely by the choices mad. EASE can be used to estimate inhalation and dermal exposure – dermal exposure is assessed as the potential exposure rate to the hands and forearms (a total skin area of approximately 2,000 cm^2). The dermal model is less developed than the inhalation model, and its outputs should be regarded as no more than first approximation estimates.

The output ranges generated by EASE relate to stead-state conditions, and estimate the average concentration of the substance in the atmosphere over the period of exposure. The model will not directly predict short-term exposures, but predictions of values for these circumstances are possible by interpreting and modifying the output data using professional judgement. Although short-term exposures may be predicted by EASE in this way, such modifications to the model output should be regarded with caution.

Some information has been made available through the manufacturers of styrene monomer, polystyrene, polyester resins etc, but detailed information regarding sampling techniques, frequency and duration of exposure were not always provided.

Where real exposure data are not available or scant, EASE has been used to predict exposures. Details of the reasoning behind any assumptions mad during the course of EASE predictions are made clear in the relevant sections.

Overview of Exposure

The total number of persons exposed to styrene in the EU is not known but because of its widespread use in UP-styrene resins, mainly in the manufacturer of glass reinforced plastic, it is expected to be thousands. During monomer and polymer manufacture throughout the EU there may be up to 1000-1200 operators exposed to styrene. In the UK there are approximately 80-100 employees exposed.

In Denmark 9200 people were working in the wind mill industry in 2003. It is estimated that about half of the workforce is occupied in the production area (Danish Working Environment Authority 2005, personal communication).

The HSE has personal exposure data on its NEDB (National Exposure Database), obtained from 1985 onwards, but relates exclusively to the glass reinforcement plastics industry. Extensive data (over 12,000 data points) have been received from CEFIC on behalf of the EU GRP industry. New data have been provided in 2005 by CEFIC relating to exposure during styrene manufacture.

There is a wealth of published exposure data, but they cover almost exclusively the use of glass reinforced plastic in manufacturing of boats, automotive products, baths and shower trays, and other miscellaneous products. Most of the published data referred to in this assessment have been collated from data published since 1990 to ensure that they are more relevant to current working practices.

The occupational exposure to styrene is discussed in five sections:

- Manufacture of monomer
- Production of polystyrene
- Production of UP-styrene resin
- Production of SBR and SB latex
- GRP manufacture

Highest exposures, were found in the manufacturing of GRP products, where the UP-styrene resin is used in the open, often with little or no effective control. Lower exposures were found where more modern semi-enclosed processes were used.

Occupational Exposure Limits

There are a number of countries with exposure limits for styrene and these have been tabulated below. These limits are provided for information and not as an indication of the level of control of exposure achieved in practice in workplaces in these countries.

Table 4.1 Occupational Exposure Limits for Styrene

Country	8-hour TWA (ppm)	STEL (ppm)	Source
UK	100	250	EH40/2005[a]
Germany	20		MAK values[b]
USA OSHA	100	200	ACGIH[c]
USA ACGIH	20	40	ACGIH[c]
USA NIOSH	50	100	ACGIH[c]
Netherlands	25		ACGIH[c]
Sweden	25	75	ACGIH[c]
Finland	20	100	ACGIH[c]
Japan	50		ACGIH[c]
France	50		ACGIH[c]
Denmark		25	**Arbejdstilsynet 2002**

(a) EH40/2005 Occupational Exposure Limits 1998, Health and Safety Executive
(b) List of MAK and BAT Values 1997, Deutsche Forschungstgemeinschaft
(c) TLV's and other Occupational Exposure Values – 2000, ACGIH Worldwide (electronic version)

In July 1993, the various European styrene-based resin producing and using industries drew up a voluntary Code of Practice to encourage the industry to work to an occupational exposure limit of 50 ppm 8-hour TWA.

4.1.1.1.2 Occupational Exposure During the Production of Monomer

Throughout the EU styrene monomer is produced in a batch process within a largely enclosed system. At one UK manufacturing site, ethylbenzene is dehydrogenated within an enclosed vessel in which benzene, toluene, ethylbenzene, residues of tar and polymers are separated to produce styrene monomer.

The styrene monomer is pumped to process units or storage tanks at 10°C ready for export. During monomer production there is venting to atmosphere. The styrene for export contains a p-tert-butyl catechol (TBC) inhibitor which prevents polymerisation. It is pumped into a road tanker from storage tanks. It takes between 30 to 40 minutes to fill one tanker. Most of the styrene produced on the site is used on site by the company to produce SBR.

At another UK manufacturing site the styrene monomer is mainly used to produce polystrene.

Industry Exposure Data

Potential airborne exposure to styrene during manufacture is limited to the collection of samples for analysis, loading/unloading of road tankers, release during venting and maintenance. At one of the production plants approximately 40 workers may be potentially exposed to atmospheric styrene. Personal sampling on the plant during road tanker loading, in the storage area, in the production area and in the laboratory during analysis has been carried out. Samples were taken using charcoal tubes, thermally desorbed and analysed by chromatography (GC). Results gave 8-hour TWA levels below 8.0 ppm. See Table 4.2

below. No information on short-term exposures was available. It was unclear from the information provided what the patterns of exposure were.

Table 4.2 8-hr TWA exposure levels during manufacture of styrene

Work Area	No. of Samples	Exposure (ppm 8-hr TWA)	
		Range	Arithmetic Mean
Styrene Plant	14	0-7.2	5.3
Styrene Storage	12	0	0
Tanker Loading	12	0-1.0	0.5
Laboratory	13	0-6.0	2

One German manufacturer of styrene reported that workplace exposure to styrene vapour is monitored regularly. The company stated that personal exposure to airborne styrene is well below 20 ppm 8-hour TWA during the production process. No further details were provided.

New industry data

In 2005 CEFIC provided some new data from manufacturing plants. The 8 hr TWA data are given in Table 4.3. There was little information provided with this data on sampling methods or the duration of sampling for the short term samples. Information from Company H states that “the data are obtained by a personal air sampling techniques (8 hour shift concentrations). A defined air volume flow is pumped through an adsorption tube, filled with activated coconut shell charcoal (method NIOSH No 1501). After desorption the substance is analysed by gas chromatography.”

Table 4.3 8-hour TWA sampling results from industry for manufacture of styrene

Company	Year	Job	N° of samples	Min (ppm)	Max (ppm)	Median (ppm)	95 %ile (ppm)
E		Production operator	7	<0.02	0.9	0.06	
		Maintenance	7	<0.02	0.9	0.06	
		Laboratory/ sampling	7	<0.02	0.9	0.06	
		Loading/ filling	7	<0.02	0.9	0.06	
B	2004	laboratory	8	0.02	0.35		
H	2000 – 2004	Production operator	258	<0.0005	8	0.003	0.4
	2000 – 2004	Maintenance	57	0.0007	0.3	0.002	0.11
	2000 – 2004	Laboratory/ sampling	126	0	1.5	0.002	0.3
	2000 – 2004	Loading/filling	52	0.0006	0.4	0.003	0.25
	2000 – 2004	Pilot plants	58	<0.0005	0.8	0.002	0.23
G	1995 -1996	monomers and rubbers	14	<0.28	0.7		
	2001- 2002	monomers and rubbers	13	0.09	2.7	0.6	2.2
	1996	maintenance	22	0.12	4.57		
	2003	Monomers and rubbers	12	<0.009	0.95		0.82
	2001 - 2002	Cleaning contractor	2	2.8	3.9		

Company	Year	Job	N° of samples	Min (ppm)	Max (ppm)	Median (ppm)	95 %ile (ppm)
J2	2004	Production operator/inspection	80	0.004	0.016	0.002	0.004
			72	0.004	0.03	0.004	0.0009
	2004	Laboratory/sampling	8	0.0009	0.0009	0.0009	0.0009
			16	0.0009	0.0009	0.0009	0.0009
			35	0.0009	0.003	0.0009	0.0009
			11	0.0009	0.04	0.004	0.005
	2004	Loading/filling	24	0.0009	0.02	0.0009	0.0009

Modelled Exposure

Inhalation Exposure

As no exposure data for short-term exposure were available, EASE has been used to predict exposure during short-term activities such as product sampling and loading/unloading of tankers. Details of sampling activities were not available, so for the purposes of modelling reasonable worst case and typical exposures the default parameters from the TGD will be used. For manufacture of a chemical, including sampling the parameters for typical exposures are no aerosol formation, non dispersive and LEV present. This results in an exposure prediction of 1 to 3ppm. For RWC (Reasonable Worst Case) exposures the parameters are no aerosol formation, non dispersive use and LEV absent. This results in an exposure prediction of 20 to 50 ppm.

As sampling is estimated to take about 2 minutes and if it is assumed that it is carried out twice per shift then the range for typical exposures would be 0.3 to 0.8 ppm and the range for the RWC would be 5.3 to 13.3 ppm. Tanker loading is reported to take 40 minutes, with the styrene stored at 10°C. This 40 minutes will consist of 5 minutes for coupling and decoupling pipework and 35 minutes standing by. If it is assumed that tanker coupling/uncoupling is carried out once per shift then the range for typical exposures would be 0.3 to 1.07 ppm and the range for the RWC would be 6.6 to 16.6 ppm.

Dermal Exposure

No measured dermal data are available and the default values given in the TGD will be used.

A typical task is the quality control sampling of liquids (incidental contact and non dispersive use) which gives a predicted exposure of 0.1 mg/cm^2/day, with an assumed exposed surface area of 210 cm^2. A RWC task would be coupling/decoupling of a transfer line. The parameters for this task are the same as sampling thus the predicted exposure would be 0.1 mg/cm^2/day but with an assumed exposed surface area of 420 cm^2.

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Values for risk characterisation

For long term inhalation exposure there are a reasonable number of real sampling results. Although there is some variation in the values, there is little background information to go with them. These results indicate that the typical exposure during this process should be 0.1 ppm 8 hr TWA and the RWC should be 1 ppm 8 hr TWA.

For short term inhalation exposures there are no real measurements and the values generated by the EASE model for coupling/uncoupling of lines will be used. The typical exposures value will be 1 ppm 15 minute TWA and the RWC will be 15 ppm.

For dermal exposure a typical value of 0.1 mg/cm^2/day over 210 cm^2 and a RWC value of 0.1 mg/cm^2/day over 420 cm^2 will be used for risk characterisation.

4.1.1.1.3 Occupational Exposure During The Production of Polystrene

Five companies produce polystyrene (GP, HI or expandable) within the UK. One such firm produces both GP-PS and HI-PS by a continuous mass polymerisation of styrene and full production of the required polystyrene is achieved within 24-48 hours. Production of the HI-PS begins with the granulation and dissolving of a number of additives into a mixture of styrene monomer and solvent. Feed mixture is fed to pre-polymerisation reactor and then into another vessel where polymerisation occurs. The polymer is pumped through a diehead after heating to form strands which are cooled in a water bath and pelletised. Depending on the grade, a lubricant may be added before conveying the product to storage and packaging facilities.

Potential airborne exposure to styrene may occur during the polymerisation process, process sampling, spillage, storage activities and maintenance. It is indicated by one company producing polystyrene that storage of the styrene monomer is in bunded tanks and delivery to the plant is by fixed line. The handling of the raw material is minimal and the possibility of leakage is low. The finished products are stored in a dedicated storage area.

Industry Exposure Data

One company stated that between 30-40 employees are potentially exposed to styrene during polystyrene production on their site. The company have sampled for personal exposures to airborne styrene during routine plant operations, sampling and general plant maintenance. Samplers were worn over a full working shift of 12 hours. Air was drawn through charcoal tubes, which were subsequently thermally desorbed and analysed by GC. All of the 8-hour shift TWA exposures were below 3.4 ppm, with an arithmetic mean of 2.1 ppm, and the short term exposure levels (15 minute reference period) were mainly below 10 ppm, although one result was 630 ppm. This high short-term exposure occurred when the operator cleared a blockage from one of the cyclone hatches. Alternative methods have now been introduced by the company and exposure during this operation is now much better controlled.

Another UK company uses styrene monomer for the production of expanded polystyrene (EPS) by a batch reaction within an enclosed system. During the manufacturing process approximately 100 employees may be potentially exposed to airborne styrene. LEV is provided at sampling points where the operator may be exposed during collection. During handling of styrene the company reported that employees use neoprene or nitrile gloves and goggles. Other activities where exposure may occur are during the loading/unloading of road tankers, working within the storage area, during venting, and extruding of the polymer. The company routinely monitors for personal exposure to airborne styrene using charcoal tubes, which are subsequently thermally desorbed and analysed by GC. The data in Table 4.4 were obtained in the mid 1980’s but the company indicates that current exposure levels for similar operations are lower. This is because operators are now multi-skilled, giving them more job flexibility and hence reducing the time spent by an individual in areas where exposure to styrene can occur.

Table 4.4 8-hour TWA exposure levels during production of polystyrene (1980s)

Job Activity	No. of Samples	8 – hour TWA (ppm)	
		Range	Geometric Mean
Plant Operator	6	0.1-4.4	0.4
Packing Operator	34	0.01-15.8	0.3
Extruder Operator	28	0.02-9.6	0.2
Shift Foreman	18	0.02-1.4	0.2
Technician	8	0.03-1.1	0.3

From the 94 samples taken, all personal occupational exposures to styrene were below 16 ppm 8-hour TWA.

UK companies reported that the occupational exposure results provided by them reflected those found in their European plants for similar operations, with most personal exposures being well below 5 ppm 8-hour TWA.

Industry have provided some new exposure data from two EU companies that manufacture polystyrene. At Company A, for routine operations styrene is handled in closed systems which are only opened for tasks like sampling and loading/unloading operations. 111 personal sampling results that were taken between 2000 and 2004 were provided for production operators. No information was given on RPE use. They were all 8 hr TWAs and were collected using 3M 3500 badges, which were analysed after solvent desorption. The range of these exposures were from 0.01 to 4.8 ppm, with a median value of 0.1 ppm and a 95th percentile value of 1 ppm.

Company provide four 8hr TWA sampling results taken during routine polystyrene production, which were 0.05, 0.05, 0.08 and 0.09 ppm. No further information was provided.

Industry reported that they expected that exposure levels for the other polystyrene plants to be very similar for routine operations. However, it must be borne in mind that higher exposures will occur, for example during plant turnarounds when equipment and reactors are being overhauled or in the vicinity of spills. At these times exposure would invariably be reduced by the wearing of PPE.

Modelled Exposure

Inhalation

The processes, and therefore likely exposures, in this scenario are similar to those during the manufacture of monomer and the same parameters for modelling will be used.

For short term exposure modelling the default parameters from the TGD will be used. For manufacture of a chemical, including sampling the parameters for typical exposures are no aerosol formation, non dispersive and LEV present. This results in an exposure prediction of 1 to 3ppm. For RWC exposures the parameters are no aerosol formation, non dispersive use and dilution ventilation. This results in an exposure prediction of 20 to 50 ppm.

As sampling is estimated to take about 2 minutes and if it is assumed that it is carried out twice per shift then the range for typical exposures would be 0.3 to 0.8 ppm and the range

for the RWC would be 5.3 to 13.3 ppm. Tanker loading is reported to take 40 minutes, with the styrene stored at 10°C. This 40 minutes will consist of 5 minutes for coupling and decoupling pipework and 35 minutes standing by. If it is assumed that tanker coupling/uncoupling is carried out once per shift then the range for typical exposures would be 0.3 to 1.07 ppm and the range for the RWC would be 6.6 to 16.6 ppm.

Dermal Exposure

No measured dermal data are available and the default values given in the TGD will be used.

A typical task is the quality control sampling of liquids (incidental contact and non dispersive use) which gives a predicted exposure of 0.1 mg/cm^2/day, with an assumed exposed surface area of 210 cm^2. A RWC task would be coupling/decoupling of a transfer line. The parameters for this task are the same as sampling thus the predicted exposure would be 0.1 mg/cm^2/day but with an assumed exposed surface area of 420 cm^2.

Values for risk characterisation

For long term inhalation exposure there are a few real sampling results, but they are from the 1980s and therefore relatively old. The company which supplied the data in Table 4.4 indicated that 'current' exposures are lower. The geometric mean values given for the 1980s are not dissimilar to the typical value for exposure during monomer production and therefore 0.1 ppm 8hr TWA will be used for risk characterisation. It is more difficult to evaluate the data for the RWC. For monomer production the RWC is 1ppm, which is based on real sampling data. Although the highest value recorded in the 1980s is approximately 16 ppm, it is likely that control will have improved since then and therefore as the process is very similar to monomer production the same RWC will be used. This judgement is backed up by the new data provided by industry from company A which have a 95th percentile value of 1 ppm from 111 sampling results. This gives a typical exposure during this process of 0.1 ppm 8 hr TWA and a RWC of 1 ppm 8 hr TWA.

For short term inhalation exposures there are no real measurements and the values generated by the EASE model for coupling/uncoupling of lines will be used. The typical exposures value will be 1 ppm 15 minute TWA and the RWC will be 15 ppm.

For dermal exposure a typical value of 0.1 mg/cm^2/day over 210 cm^2 and a RWC value of 0.1 mg/cm^2/day over 420 cm^2 will be used for risk characterisation.

4.1.1.1.4 Occupational Exposure Data During Production of UP-styrene Resin

Most commercial thermosetting polyester resins are formed by the reaction of a mixture of unreactive and reactive (saturated and unsaturated) dibasic acids with a dihydric alcohol such as ethylene glycol. The reactive acid component is almost always maleic acid and the unreactive acid is mainly phthalic acid. Styrene is the reactive cross-linking monomer in which the polyester is readily soluble. The content of styrene monomer in the uncured resin is generally in the region of 40%.

Several companies manufacture UP-styrene resin in the EU for use in the building, transport, marine and paint industries.

Industry Exposure Data

One major supplier of UP-styrene resin produces the polymer within a largely enclosed system. Styrene is purchased in bulk and stored in a holding tank. Dibasic acids and glycols are reacted in a blender and polymerisation occurs. The resultant polymer is dissolved in styrene in a different blender. There may be some styrene vapours released during the blending process but these are controlled by LEV. After blending, the UP-styrene resin is run-off into 200-kilo drums or occasionally 1 tonne tanks or road tankers. Occupational exposure to styrene may occur during discharge from the blender into storage or when drumming off. However the filling point above the drum is provided with LEV and the employees generally wear respiratory protective equipment. The number of drums filled per day varies and is dependant on market demand but it is rarely less than four.

Background monitoring has been carried out during UP-styrene resin manufacture. The company installed styrene monitors on the blender to measure background short-term exposure when samples are taken for analysis. This operation takes approximately 1-2 minutes. The data in Table 4.5 were obtained using static sampling over a 10-minute period. All short-term measurements were below 50 ppm.

Table 4.5 Occupational Exposure during production of UP-styrene resin (background monitoring)

Area	Short term measurements over a 10 min reference period (ppm)	
	Range	Arithmetic Mean
Reactor Charging	1-5	2
Blending Area	1-8	4
Reprocessing	2-5	3
Drumming Off	2-46	10

Operators were monitored for personal exposure to airborne styrene during the drumming-off of resin. Samples were collected using charcoal tubes, which were thermally desorbed and analysed by GC. All exposures ranged between 2-6 ppm 8-hour TWA.

A second firm producing resin for the production of caravans, car accessories and small boats purchases styrene in 18 tonne tankers and stores the styrene in dedicated storage vessels from where it is pumped to a blender and mixed with the appropriate additives. The UP- styrene resin is then piped to holding tanks and discharged into drums for transport to customers. On a busy day approximately 100 drums may be filled and almost 15-20 employees potentially exposed to atmospheric styrene. The company perceives the risks from airborne styrene exposure to be minimal and hence operatives handling the substance wear no respiratory protection although they stated that employees did wear gloves and eye protection. Personal exposure monitoring by the company in the main resin producing room and in the resin formulating plant indicate that exposure to airborne styrene is less than 1.0 ppm 8-hour TWA. No further details were provided by the company.

Modelled Exposure

Inhalation

EASE was used to predict short-term exposure sampling at the blender, the task likely to lead to the highest exposures. The TGD default parameters for production of liquid formulations are no aerosol formation, non dispersive use and LEV present for typical exposures and no aerosol formation, non dispersive and dilution ventilation for RWC exposures. This results in a typical exposure range of 1 to 3 ppm. As sampling is likely to take 2 minutes and be carried out twice in a shift the 15 minute TWA for this task is 0.3 to 0.8 ppm. The predicted RWC range is 20 to 50 ppm and if a task duration of 2 minutes is taken into account the 15 minute TWA is 5.3 to 13.3 ppm.

EASE was also used to estimate exposure during drumming off. The parameters used for typical exposures were non-dispersive use with LEV, which results in a prediction of 1 to 3ppm. For the RWC exposures the parameters used were no aerosol formation, non dispersive use and dilution ventilation, which results in a prediction of 20 to 50 ppm.

Dermal Exposure

The main potential for dermal exposure is likely to occur during drum filling. The EASE parameters best suited to typical exposures for this scenario are non-dispersive use, direct handling with incidental contact with a liquid. The predicted dermal exposure range was 0 to 0.04 mg/cm^2/day over 210 cm^2, taking into account that the resin contains 40% styrene monomer. A reasonable worst-case exposure for this scenario for dermal exposure would be non dispersive use with intermittent exposure. The RWC value is therefore 0.4 mg/cm^2/day over 210 cm^2, taking into account that the resin contains 40% styrene monomer.

Values for risk characterisation

The task with the highest potential for exposure is drumming of the liquid polystyrene and the values for this task will be used for risk characterisation.

For long term inhalation exposure there is a small amount of real sampling data, but it has little contextual information accompanying it. These data indicate that exposures during drumming are below 1 ppm or between 2 and 6ppm. The typical values given by EASE range from 1 to 3 ppm. Therefore a value for typical exposures of 3ppm 8 hr TWA will be used for risk characterisation. For the RWC EASE predicts a range of 20 to 50 ppm. As it is more likely that some form of control will be available at drumming and the operator is unlikely to spend the whole shift at the drumming point, the lower of these two values, 20 ppm 8 hr TWA will be used for risk characterisation.

For short term inhalation exposure there are a few static measured data, which range from 2 to 46 ppm, sampled over 10 minutes, which presumably were taken near to the drumming point. The predicted EASE range for this task is 20 to 50 ppm. If the operator were to spend 15 minutes next to the drumming point their exposure could be as high as 50 ppm, therefore the value of 50 ppm 15 minute TWA will be used as the short term RWC. The short term typical value will be based on three times the long term typical value, which results in a short term typical value of 9 ppm 15 minute TWA.

For dermal exposure the typical exposure value for this scenario is 0.04 mg/cm^2/day over 210 cm^2. The RWC for this scenario for dermal exposure is 0.4 mg/cm^2/day over 210 cm^2.

4.1.1.1.5 Occupational Exposure Data During SBR and SB Latex Production

In the EU almost 40 Companies produce SBR, ABS, SAN and SB latex formulations. SBR is produced by manufacturing companies throughout the EU in a closed system using 25% styrene and 75% butadiene; there may be slight variations in the percentage of each substance used depending on customer specifications. Both the styrene and the butadiene are copolymerised in a continuous polymerisation process initiated by a hydroperoxide catalyst. The polymerisation is terminated by the addition of sodium polysuphide or sodium dimethyl dithiocarbamate, and unreacted butadiene and styrene is recovered from the latex in the monomer recovery area. The SB latex is then either converted to solid rubber or used as a feedstock to produce high solid lattices.

Industry Exposure Data

One UK firm producing SBR indicated that 5 employees may be potentially exposed to airborne styrene during the production process with the highest exposure occurring during monomer recovery, sampling and venting to atmosphere. Personal sampling during these activities using charcoal tubes, which were subsequently solvent desorbed and analysed by GC gave exposure levels to airborne styrene of up to 6.5 ppm 8-hour TWA with the majority below 2.5 ppm 8-hour TWA. No further information was provided.

SB latex and ABS are produced by one UK manufacturer for use in carpet backings and in the paper industry. During SB latex production the monomer is transferred automatically via pipeline from a storage tank to a reactor where the appropriate chemicals are mixed. The polymerisation reactions occur in a range of glass-lined reactors and the system is enclosed. Following completion of the polymerisation reactions, the batch is transferred to holding vessels. The SB latex is sieved to remove coagulum before despatch to customers in road tankers. On site, approximately 20 to 25 employees are potentially exposed to airborne styrene especially during the coupling/uncoupling of the road tankers, venting and sampling. The employees are routinely monitored for personal exposure using passive samplers with charcoal, which are solvent desorbed and analysed by GC. The company has also undertaken a series of background monitoring for airborne styrene at different locations on the site using colorimetric indicator tubes. Personal exposures and background concentration of less than 5 ppm 8-hour TWA have been obtained with the majority of concentrations being less than 1.0 ppm 8-hour TWA. In one study, in 1993, workers were sampled for personal exposure to styrene using charcoal tubes during routine operations, unloading of styrene monomer from tanker to storage tank, and during routine operations on the plant manufacturing site. Most exposure levels were $<$1.0 ppm 8-hour TWA but during maintenance operations exposures of 10 ppm 8-hour TWA were obtained. No further information was provided.

In 2005 CEFIC provided some new sampling data. These results are presented in Table 4.6. They were all taken at one company in 2003/4 and are all 8 hr TWAs. No further information was provided.

Table 4.6 8 hr TWA sampling results SBR latex production.

Job	No of samples	Min (ppm)	Max (ppm)	Median (ppm)	95 %ile (ppm)
Production operator/inspection	43	0.01	1.2	0.12	0.5
Laboratory/sampling	27	0.01	0.25	0.06	0.18

SBR polymerisation operator	23	0.01	2.5	0.35	2.3
SBR monomer recovery	26	0.17	2.7	1.25	2.6
SBR finishing operator	42	0.02	0.5	0.2	0.5
Latex production operator	54	0.01	0.56	0.09	0.19

Modelled Exposure

Inhalation Exposure

Information to enable EASE to be used to model short-term exposures was not available, so the same scenarios are used in the monomer production sections are used here.

EASE has been used to predict exposure during short-term activities such as product sampling and loading/unloading of tankers. Details of sampling activities were not available, so for the purposes of modelling reasonable worst case and typical exposures the default parameters from the TGD will be used. For manufacture of a chemical, including sampling the parameters for typical exposures are no aerosol formation, non dispersive and LEV present. This results in an exposure prediction of 1 to 3ppm. For RWC exposures the parameters are no aerosol formation, non dispersive use and LEV absent. This results in an exposure prediction of 20 to 50 ppm.

As sampling is estimated to take about 2 minutes and if it is assumed that it is carried out twice per shift then the range for typical exposures would be 0.3 to 0.8 ppm and the range for the RWC would be 5.3 to 13.3 ppm. Tanker loading is reported to take 40 minutes, with the styrene stored at 10°C. This 40 minutes will consist of 5 minutes for coupling and decoupling pipework and 35 minutes standing by. If it is assumed that tanker coupling/uncoupling is carried out once per shift then the range for typical exposures would be 0.3 to 1.07 ppm and the range for the RWC would be 6.6 to 16.6 ppm.

Dermal Exposure

Again, during production, the main tasks where there is a potential for dermal exposure are during sampling and coupling and uncoupling of pipework during tanker loading. No measured dermal data are available and the default values given in the TGD will be used.

A typical task is the quality control sampling of liquids (incidental contact and non dispersive use) which gives a predicted exposure of 0.1 mg/cm^2/day, with an assumed exposed surface area of 210 cm^2. A RWC task would be coupling/decoupling of a transfer line. The parameters for this task are the same as sampling thus the predicted exposure would be 0.1 mg/cm^2/day but with an assumed exposed surface area of 420 cm^2.

Values for risk characterisation

For long term inhalation exposure there are a reasonable number of real sampling results, but most are all from one site and so there is little indication of how representative they are. The other available long term data indicate that 8 hr TWAs are below 6.5 ppm, with the

majority below 1 ppm. Taking all of this into account, it is judged that the typical exposure during this process should be 1 ppm 8 hr TWA and the RWC should be 5 ppm 8 hr TWA.

For short term inhalation exposures there are no real measurements and the values generated by the EASE model for coupling/uncoupling of lines will be used. Therefore a RWC of 15 ppm 15 minute TWA and a short term typical value of 1 ppm 15 minute TWA will be used for risk characterisation.

For dermal exposure a typical value of 0.1 mg/cm^2/day over 210 cm^2 and a RWC value of 0.1 mg/cm^2/day over 420 cm^2 will be used for risk characterisation.

4.1.1.1.6 Occupational Exposure Data During GRP Manufacture and UP-styrene use

In the GRP industry, styrene exposures are higher than those found in monomer and polymer production. This is because the processing of the styrene-containing resins involves manual handling in either open or semi-closed moulding processes.

Styrene is used as a cross-linking agent and during fabrication methyl ethyl ketone peroxide is used to initiate the exothermic polymerisation. Other substances are added as regulators and stabilisers. The GRP is a laminate consisting of the UP-styrene resin matrix and glass fibre.

In the GRP industry, there are two categories of fabrication, namely contact moulding and machine moulding. Machine processes are generally used for the production of small to medium sized items such as containers, sheets and tubes. Contact moulding operations are often manual and can involve hand lay-up or spray up lamination to deposit UP-styrene resin and fibre glass onto the surface of a prepared mould. In hand lay-up the UP-styrene resin is applied direct to the laminate by spraying and then covering with fibreglass mat by hand. In spray-up, both the resin and chopped fibreglass are applied simultaneously to the mould. The laminate is then rolled out manually to remove any entrapped air bubbles. The operations are repeated until the correct thickness is obtained. After a few hours, curing is usually complete. Edges may be trimmed during or after the curing process.

With these open moulding techniques the operators work very near the moulds and there is considerable potential for exposure to evaporating styrene. The concentration of styrene in the working atmosphere will vary according to the amount of resin used, the area of surface fabrication, duration of work process, temperature and whether LEV is provided. Often, work practices are poor, with operators leaning over large work pieces or working between the workpiece and LEV, thus rendering any LEV present ineffective. In open moulding up to 10% of the styrene contained in the resin may volatise into the workplace and, without appropriate controls, airborne exposures can be high.

There is an increasing use of semi-enclosed processes such as the RTM (Resin Transfer Moulding) process, and sheet and bulk moulding compound (SMC & BMC) processes. These modern techniques can significantly reduce exposure as they reduce the time the operator is in contact with the resin. Traditional hand and spray lay-up techniques require greater operator input to work the mould, usually with hand lamination. This may require the operator to be leant over the mould whilst using hand tools to work the resin into the mould.

The RTM method utilises an enclosed mould where the operator's hand lay-up work is replaced by the upper half of the mould. The resin is fed in from one side of the mould using pressure or vacuum injection and surplus resin is removed from the opposite side. A similar system can be retrofitted to an existing hand lay-up process. A new mould table needs to be purchased, which incorporates a vacuum injection system. The top half of the mould is

plastic sheeting, which is laid-over the reinforcing material. The resin is injected into the reinforcing material between the bottom solid half of the mould and upper sheeting. This removes the need for the operator to hand work the resin into the mould. Many companies move to this method as a cheaper alternative to the RTM process. There are other semi-enclosed techniques, such as sheet and bulk moulding compound, which can also reduce exposures.

Occupational exposures can still occur with these semi-enclosed processes during mould opening/closing when residual styrene is released. Other activities, such as connecting resin delivery lines or maintenance may also result in workers being exposed to styrene. However, these semi-enclosed resin moulding processes do provide a significant improvement over open moulding techniques. Open moulding techniques will suffer higher exposures due to the high level of operator input needed to work the mould, and are dependent on a greater level of operator training to manage these exposures. Techniques, such as RTM etc., which remove the high level of operator input will therefore reduce exposures.

Low styrene emission (LSE) resins will also help to reduce exposures. It is reported that these resins can have problems with delamination between layers, but this problem has now been largely overcome and they are used extensively, including by the boat building industry.

Industry Exposure Data

One medium sized company employs 150 workers and uses styrene monomer in a UP-styrene resin with methyl ethyl ketone peroxide as a catalyst for polymerisation. The UP-styrene resin and glass fibre filaments are used to produce building and housing panels and during the lamination process up to 50 employees may be potentially exposed to airborne styrene. The process is semi-automated contact moulding and resin is automatically mixed with the catalyst prior to being sprayed onto the mould surface along with the glass fibre strands. Once deposited, the laminate is rolled by 4 – 5 employees using brushes and it is left to cure into its finished state. The lamination is carried out in 2 booths with installed LEV and approximately 12 –15 panels are completed in one day. Measurements of personal exposures to styrene indicate that 8-hour TWA's are below 100 ppm. The personal samples were taken during laminate spraying and rolling in the breathing zone of the operatives using charcoal tubes, which were subsequently thermally desorbed and analysed by GC. The results are shown in Table 4.6

Table 4.6 Industry data on exposure to styrene during GRP manufacturing

Activity	Samples	Exposure 8-hour TWA (ppm)	
		Range	Arithmetic Mean
GRP Laminate Sprayer	24	6 to 46	13.3
GRP Laminate Roller	18	7.5 to 85	44.2

Industry Exposure Data (received from CEFIC)

The following occupational exposure data were received from CEFIC. These data were collated from member states by CEFIC and the following tables (reproduced directly from their report) were produced to summarise exposures across the range of activities using

GRP. All of the exposure values are in ppm and were taken using a variety of methods. They were taken over different time periods and may not all represent 8-hour TWA's. The report provided the following conclusions.

- Open mould operations show the highest exposure of workers to styrene with gel coating being the activity with the highest exposure. The wealth of data on open mould operations show that keeping the workers exposure below the specified MAC value has proved to be very difficult.

- Conversely in all data coming from closed mould injection operations the styrene concentration stays well below the MAC value.

- SMC/BMC operations also typically show a high variation in styrene concentrations. The highest values are obtained during SMC moulding when SMC sheets are laid in the press, essentially the "open mould" part of the operation.

Table 4.7 Hand Lay Up

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Austria	19	2.6	52.1	17.1	8.6	37.3
Belgium	3	4.9	41.2	24.4	*	*
Denmark	156	0.3	282.7	31.9	19	73.6
France	1500	0.2	313.8	45.2	36.3	101
Germany	1171				18	61
Italy	1030			55.4	38.6	
Netherlands	48	4	111	31	22	68
UK	255	1	374	35	23	90

N – number of samples * Insufficient data

Table 4.8 Spray Up

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Austria	1	11.7	11.7	11.7	*	*
Belgium	23	0.7	84.5	18.9	10.6	47.6
Denmark	3	49.1	186.9	105.9	*	*
France	458	2.3	380.1	43.6	41.7	98
Italy	166			31.3	19.2	
Netherlands	76	2	374	46	30	94
UK	1	45	45		*	*

N – number of samples * Insufficient data

Table 4.9 Gel Coating

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)

Austria	4	8.2	13.7	11.5	*	*
Belgium	2	9.9	50.5	30.2	*	*
Denmark	36	3	133.2	28.8	19.6	60.8
France	259	0.1	193.2	31.3	21.7	85
Germany	67				10.7	47.9
Netherlands	12	9.2	100	47.3	34.9	98
UK	2	26	45	35.5	*	*

N – number of samples * Insufficient data

Table 4.10 RTM

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Denmark	33	0.5	49.1	3.8	1.8	5.7
Sweden	1	0.5	0.5		*	*
UK	19	1	98	16.3	8	27.8

N – number of samples * Insufficient data

Table 4.11 SMC/BMC Production

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Austria	1	12.9	12.9	12.9	*	*
Belgium	1	2.6	2.6			
Germany	77				17.1	38.3
UK	84	1	122	27	18	62.4

N – number of samples * Insufficient data

Table 4.12 SMC/BMC Moulding

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Austria	5	8.9	16.9	21.1	11.9	15.3
Belgium	3	6.3	56.1	28.5	*	*
France	432	0.9	290.9	44.4	33.8	102
UK	38	1	100	15	10	35.8

N – number of samples * Insufficient data

Table 4.13 Filament Winding

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Denmark	3	10.3	19.6	15.8	*	*
Italy	71			20.1	11.1	
Netherlands	20	2.1	31.0	10.3	6.4	21.6

N – number of samples * Insufficient data

Table 4.14 Pultrusion

Country	N	Min (ppm)	Max (ppm)	Average (ppm)	50%ile (ppm)	90%ile (ppm)
Denmark	20	0.2	5.8	1.6	0.8	4.1
Italy	71			20.1	11.1	
UK	23	1	68	11	7	19

N – number of samples

There are over 12,000 results in total from across most EU Member States. They generally date back over the last ten years (the French data were from 1987 to 2001) and were taken using a variety of methods. They were taken over different time periods and may not all represent 8-hour TWA's. It is therefore not possible to be confident about the quality of all the data. In addition, the data were sent to CEFIC in many different forms, some summarised and some as individual data points. In addition, to produce the above tables they had to make assumptions about the data, and in some cases generate figures for their calculations.

Whilst Table 4.10 to 4.14 generally show lower exposures, the data in Tables 4.7 to 4.9 do show that higher exposures do still take place. Modern resin moulding techniques are clearly helping to drive down exposures, but older open moulding techniques are still in extensive use across the EU. These open moulding processes result in higher exposures

Cefic Pilot Study for a Harmonised Monitoring Programme

In 2002 Cefic set up a pilot study to test a proposed harmonised monitoring programme for occupational exposure to styrene in the GRP industry. The pilot study took place in seven EU countries: Belgium and the Netherlands, Denmark, Finland, France, Italy and the UK. In each country the main resin supplier was asked to distribute 50 'do-it-yourself' sampling kits among 15 – 25 of their customers. Each 'styrene sampling kit', consisted of a 3M badge, an instruction leaflet for the worker on how to use the badge, a task registration form and a return envelope. All of the returned badges were collected and sent to the same laboratory for analysis. The study was carried out between December 2002 and April 2003.

78% of the 88 companies that received sampling kits participated in the study and 76% of the 300 sampling kits were returned. The report indicates that the duration of the measurements ranged from 400 minutes to 600 minutes, with an average sampling time of 480 minutes, therefore the results below will be assumed to be representative of 8hr TWAs. Table 4.15 gives the sampling results from this study. As has been seen in the other industry data presented, rolling and hand lamination gives rise to the highest exposures, closely followed by other open mould tasks.

Table 4.15 Cefic data on styrene concentration in breathing zone by job category

Job category	Styrene concentration in breathing zone (8hr TWA)			
	Number of samples	Min (ppm)	Max (ppm)	Median (ppm)
Gel coating	10	6.2	78	33
Spray up	17	8.3	126	44
Rolling/ Hand laminating	61	1.6	167	47
Filament winding	2	42	121	
Variable – open mould	63	2.3	112	35
Finishing	8	1.1	50	27
Injection	9	3	18	10
Cold press	4	7.8	31	15
SMC/BMC moulding	4	0.7	25	21
Variable – closed mould	5	6.2	19	9
SMC/BMC production	5	14.3	25	20
variable- other	26	0.5	44	18
Unknown	4	4	45	20
TOTAL	**218**	**0.5**	**167**	**30**

In the study report data were also presented showing the results on rolling/hand laminating by country. These data are presented in Table 4.16. There does not appear to be any appreciable difference between exposure levels in different countries for this task/job.

Table 4.16 Cefic data on styrene exposures during rolling/hand laminating by country.

Country	Styrene concentration in breathing zone Rolling/hand laminating (8hr TWA)			
	Number of samples	Min (ppm)	Max (ppm)	Median (ppm)
Belgium/Netherlands	6	25	108	55
Denmark	14	1.6	167	24
Finland	16	8.3	106	56
France	3	21.6	106.5	77
Italy	18	6.2	130.6	35

UK	4	48	93	55
Total	**61**	**1.6**	**167**	**47**

HSE Exposure Data

Personal exposure data were obtained from HSE's National Exposure Database (NEDB) for 8-hour TWA exposures to styrene vapour between 1985 and 2002 in GRP manufacturing industries. The work is described as hand lamination and included such work categories as laminators, rollers, process workers and those applying the gel coat to the mould. GRP production is by hand lay-up, spray lay-up or a combination of both. The number of workers directly exposed to styrene vapour ranges from between 5 and 50 per site. The results are summarised in Table 4.17.

Exposures reported to the National Exposure Database generally represent the worst-case scenarios, as the monitoring work is usually undertaken as a reaction to perceived problems within a company or industry. This can lead to a bias in the level of exposure recorded on the HSE database.

Table 4.17 Occupational exposure to styrene during GRP manufacturing recorded on HSE's database

Activity	Year	No. of Samples	8-hour TWA (ppm)			
			Range	Arithmetic Mean	Median	90th percentile
Hand Lamination	1985	26	4 - 158	51.4	43	102.3
	1986	65	4 - 89	37.3	30	80
	1987	180	1 - 198	55.3	43	124
	1988	15	0.25 - 109	35.3	30	90.4
	1989	36	2.2 - 68	25.5	22.5	53.2
	1990	28	3.5 - 61	24.2	21.1	44.6
	1991	35	12.5 - 75	39	39	63
	1992	48	0.6 - 97	26.5	20	57.4
	1993	11	10 - 40	19.8	20	37.4
	1994	49	2.2 - 126	45.3	43	97
	1995	11	3 - 100	34.3	26	95.6
	1996	37	13 – 69.6	39.4	34	66.8
	1997	6	42.8 – 61.3	49.4	45.5	
	1998	6	2.9 – 35.5	15.5	11	
	1999	22	25 - 105	47.6	41.5	82.3
	2000	15	0.8 - 72	28.1	19	64.8
	2001	13	23.7 – 52.3	42	44	51.8
	2002	2	13.2 - 69			

Published Exposure Data

There is a wealth of exposure data provided for GRP manufacturing. Data has been taken from papers published between 1992 and 1997, to reflect more recent working practices. The results are summarised in Table 4.18.

As can be seen from the summary table of results from published papers, in the majority of cases, average exposures were below 50 ppm 8-hour TWA. Where high results did occur, they were not usually explained by the authors, as the primary object of the published research was not to investigate levels of exposure to styrene. There were few details of the controls in place, apart from in Anderson *et al,* (1993), where the object of the paper was to demonstrate the level of control of styrene vapours achieved by a horizontal displacement ventilation system in the workplace (4.7 to 11 ppm 8-hour TWA), and Geuskens *et al,*(1992) and Lof *et al,* (1993), both of which indicated general ventilation was in place.

The results from the study by Kawai *et al* (1993) (75 to 200 ppm 8-hour TWA) were found to be high relative to the other studies because the eight workers selected had been chosen by the authors for the study from a group of twenty-eight workers previously monitored, because of their high exposure to styrene. These results can therefore be discounted when looking at representative levels of exposure for the industry. However they do demonstrate the levels of exposure which can occur when control and/or working practices are poor.

Table 4.18 Summary of published data on occupational exposure to styrene during GRP manufacturing

Source	No. of Samples	Work Activities	Range (8-hour TWA ppm)	*Mean (8-hour TWA ppm)	Controls	Method of Sampling/Analysis
Geuskens et al (1992) Holland	258	Various	-	***4 to 49***	General Ventilation	Passive, Desorp. GC
Pekari et al (1994) Finland	401	Various	-	35 to 37	No details	Passive, Desorp. GC
Kawai et al (1993) Japan	16	No Details (producing bathtubs)	75 to 200	447	No details	Passive, no details of analysis
Lof et al (1993) Sweden	20	Lamination (boat manufacture)	9 to 25	16	General Ventilation	Charcoal tubes GC
Andersson et al (1993) Sweden	4	Lamination (boat manufacture)	4.7 to 11	7	Horizontal Displacement Vent	Charcoal tubes GC
Ghittori et al (1997) Italy	22	Laminating & Spraying	10 to 53	26	No details	Passive, Desorp, GC
Severi et al (1994) Belgium	208	Laminating & Spraying	0.5 to 25	7	No details	Charcoal tubes/ Passive, GC
Gobba et al (1993) Italy	211	Various	0.6 to 178	20**	No details	Passive Desorp, GC

* Arithmetic mean unless otherwise stated

** Geometric mean
*** Range of geometric means for a variety of activities

Further data has recently been published in a paper by Kolstad et al, in 2005. 2,454 of the personal styrene exposure measurements in this study were collected by the Danish National Institute of Occupational Health between 1960 and 1996. 600 measurements came from company surveillance programmes. 87% of the available measurements were from manual lamination.

The pre 1970 long term samples were taken using an impinger method and were not included in the study analysis. After 1970 a personal sampling technique was used, where air was drawn through an absorbent layer of charcoal and analysed by gas chromatography. No further detail of the sampling method/s were given. No information was given as to what actual sampling times were or what, if any, control measures were in use.

The companies involved in the project produced boats, containers, windmill wings, panels etc. Some of the companies did not produce reinforced plastics but metal products or thermoplastics. Hand or spray lamination was the preferred production process. Between the 1960s and the 1990s levels of styrene exposure declined by 7% annually.

Table 4.19 shows the long term styrene levels in the Danish reinforced plastics industry from 1960 to 1996.

Table 4.19 Long term styrene exposure levels in Danish reinforced plastics industry 1960 – 1996 (adapted from Kolstad et al)

	Number of companies	Number of samples	Geometric mean (ppm)	Min (ppm)	Max (ppm)
Boats	45	247	39	0.9	266
Other products	80	603	18	nd	410
Hand or spray lamination	118	768	23	nd	410
Other processes	7	82	17.5	0.9	106
Calendar year period					
<1970	-	-	-	-	-
1970 - 1974	13	48	67.6	15	151
1975 - 1979	22	120	31.3	1.1	187
1980 – 1989	92	616	22.2	nd	410
1990 – 1996	2	66	6.8	0.23	90

Although the number of long term samples taken in this study declined from 616 between 1980 and 1989 to 66 between 1990 and 1996, a substantial drop in the levels to which workers were exposed by the 1990s can be seen. Again the highest exposures were for

hand lamination, with exposures for 1990 to 1996 ranging from 0.23 to 90 ppm, with a geometric mean of 6.8 ppm.

Short term exposures

There are relatively few short term sampling data available. Some information is given in Kolstad et al and is summarised in Table 4.20. The highest levels were seen at the earlier part of the study and maximum levels show a decrease from 626 ppm before 1970 to 168 ppm between 1990 and 1996. Although this level is for all processes, and not just hand lamination, it is felt to be more representative of current practices than the specific data for hand lamination, which includes all of the old data.

Table 4.20 Short term styrene exposure levels in Danish reinforced plastics industry 1960 – 1996 (adapted from Kolstad et al)

	Number of companies	Number of samples	Geometric mean (ppm)	Min (ppm)	Max (ppm)
Boats	63	670	61.5	nd	626
Other products	125	1537	24	nd	552
Hand or spray lamination	179	2074	32	nd	626
Other processes	9	133	24	0.7	173
Calendar year period					
<1970	14	113	120	11	626
1970 - 1974	31	425	62.5	2.3	575
1975 - 1979	37	360	46	0.9	395
1980 – 1989	121	954	24	nd	447
1990 – 1996	2	355	13	0.23	168

HSE's NEDB has a small number of short term sampling data taken from 1990 to 2002 during GRP manufacture. These are shown in Table 4.21 .

Table 4.21 HSE short term sampling data 1990 to 2002

Activity	Number of samples	Min (ppm)	Max (ppm)	Median (ppm)	90th %ile (ppm)
Hand lay-up/ lamination	30	10	219	69	145
Gel coating	10	4	123	52.5	123
Spray up	2	60.5	62.7		
All activities	42	4	219	60.25	141.5

EASE can be used to predict task based exposures for hand lay-up /lamination. Using the parameters wide dispersive use and direct handling with dilution ventilation, EASE gives a predicted exposure range of 140 to 200 ppm.

Dermal Exposure

There are few dermal exposure measurements available for the GRP industry. The Riskofderm project had a scenario (3.4) which was relevant to this industry and collected data on the application (spreading) of styrene by rolling. Rolling was studied at enterprises where plastic boats for leisure activities were produced. The duration of the scenario was in general between 35 to 110 minutes and the formulation used contained approximately 40% styrene, 60% of styrene resin and a few % of the hardener ethyl methyl ketone peroxide. The potential exposure of the hands was evaluated by attaching a cotton/charcoal patch at the inner and outer side of cotton gloves. The results in the final report of Workpart 3 : Annex II are given in the same format as in the TGD. For both the RWC and the typical value the exposed skin surface area is assumed to be 820 cm^2 . The RWC value given is 8 mg/cm^2 and the typical value is 1.2 mg/cm^2.

Modelled Dermal Exposure

As GRP manufacturing involves mostly manual work in close proximity to the UP-styrene resin for long periods of time, the EASE parameters used to estimate dermal exposure were wide-dispersive use, direct handling with extensive contact with a liquid containing 40% styrene monomer. The estimated range of dermal exposure was 5 to 15 mg/cm^2/day. Taking into account that there is only 40% styrene monomer in the resin, the dermal exposure range becomes 2 to 6 mg/cm^2/day, with an assumed exposed area of 840 cm^2.

Values for risk characterisation

Inhalation

Long term

A reasonably large quantity of data has been presented with respect to occupational exposure in the GRP industry. Much of it lacks a reasonable level of contextual data to allow the exposure levels in different studies to be directly comparable. Table 4.22 summarises the most pertinent data from all of the data presented above relating to hand lay-up/lamination which is regarded as the job/task which usually leads to the highest levels of exposure. It is these data which were the main basis for the determination of the 8hr TWA RWC and the typical values. Only the most recent data have been be included.

Table 4.22 Summary table of 8hr TWA exposure data for hand lay up/lamination used in determining RWC and typical values for inhalation exposures

Source	Year	Number of samples	Min (ppm)	Max (ppm)	Median (ppm)	90th %ile (ppm)
Original CEFIC report		4182	0.1	380	8.6 – 38.6	37 - 101
Cefic Pilot study for harmonised monitoring programme	2002/2003	61	1.6	167	47	
HSE data	1997	6	42.8	61.3	45.5	
	1998	6	2.9	35.5	11	
	1999	22	25	105	41.5	82.3
	2000	15	0.8	72	19	64.8
	2001	13	23.7	52.3	44	51.8
	2002	2	13.2	69		
Kolstad et al (published 2005)*	1990 – 1996	66	0.23	90	6.8**	90

* the most recent data on all processes is used as the specific hand lamination data includes some very old data.
** geometric mean

Evaluation of this data leads to the conclusion that the 8hr TWA RWC should be 100 ppm and the typical 8 hr TWA should be 40 ppm

Short term

There are very few data available on short term exposures. Taking into account the data from Kolstad et al , data from HSE's NEDB and the EASE prediction, it is proposed that the short term (15 minutes) RWC for the GRP industry should be 180 ppm and the short term (15 minute TWA) typical value should be 60 ppm.

Dermal

There are some dermal exposure measurements available for this scenario from the Riskofderm project and these will be used for risk characterisation as they are based on real measurements. The estimates produced by EASE are very similar. Therefore the dermal RWC is 8 mg/cm^2, over 820 cm^2 and the typical dermal value is 1.2 mg/cm^2 over 820 cm^2.

4.1.1.1.7 General discussion

Exposure to styrene can be considered in two main categories; manufacturing of monomer, polymer and copolymers, and GRP manufacture.

As can be seen the exposure to employees in the industries producing monomer, polystyrene, UP-styrene resin and SBR and SB latex is much lower than that experienced by employees in the GRP manufacturing industry. Manufacturing processes are largely

enclosed with breaches for sampling, drum filling, and coupling and uncoupling of pipework for tanker loading. Where breaches occur, exposure is often controlled by the use of LEV. The level of exposure experienced during maintenance activities is potentially one of the highest. The use of respiratory protective equipment is also sometimes used during breaches in the system, which should further reduce actual exposure to styrene.

Within the GRP industry in the EU, there is a much wider range of exposures experienced by workers. In HSE's database there is a range of 0.6 to 158 ppm 8-hour TWA. Results originally supplied by CEFIC showed exposures of between 0.1 and 380 ppm 8-hour TWA. More recent data supplied by CEFIC, taken using a standardised sampling method, range from 1.6 to 167 ppm 8hr TWA. The higher exposure experienced by the GRP manufacturing industry compared to the other users of styrene is a reflection of the work methods, which in many case are open, labour intensive processes. More modern semi-enclosed processes are now available and are used more significantly by some member states than others, but the use of open moulding techniques is still widespread. The size of the companies also varies from about 5-50 employees, with the smaller companies exhibiting lack of awareness of the need to control exposure and the methods by which to achieve control.

There has been an overall reduction in workplace exposure to styrene during the last decade as better controls have been implemented. Semi-enclosed moulding processes remove the need for hand laminating and do reduce exposures.

The potential for dermal exposure in the monomer, polymer and copolymer group of industries occurs during breaches in largely closed systems; during sampling, drum filling and coupling and uncoupling of pipework during tanker loading. Any differences in the predicted ranges of exposure are due to the percentage content of styrene monomer in the substances being manufactured. Almost all companies in these industries reported the use of PPE during breaches in the enclosed system of manufacture.

The Riskofderm project provided some real dermal exposure measurements for the GRP scenario. The dermal RWC is 8 mg/cm^2, over 820 cm^2 and the dermal typical value is 1.2 mg/cm^2 over 820 cm^2. This reflects the open nature of the process and the large degree of manual handling of the up-styrene resin required. The EASE prediction for dermal exposure gives similar values.

A summary of all of the values used in risk characterisation is given in Table 4.23 below.

Table 4.23 Summary table of occupational exposures to styrene taken forward to risk characterisation

SCENARIO	INHALATION						DERMAL mg/cm^2/day (area exposed)		
	8- HOUR TWA ppm *(mg/m^3)*			SHORT TERM ppm *(mg/m^3)*					
	TYPICAL	RWC	SOURCE	TYPICAL	RWC	SOURCE	TYPICAL	RWC	SOURCE
Manufacture of monomer	0.1 (0.433)	1 (4.33)	Industry	1 (4.33)	15 (65)	EASE	0.1 (210)	0.1 (420)	EASE
Production of polystyrene	0.1 (0.433)	1 (4.33)	Industry	1 (4.33)	15 (65)	EASE	0.1 (210)	0.1 (410)	EASE
Production of UP-styrene resin	3 (13)	20 (86.6)	Industry/EASE	9 (39)	50 (216.5)	EASE	0.04 (210)	0.4 (210)	EASE
Production of SBR and SB latex	1 (4.33)	5 (21.65)	Industry/EASE	1 (4.33)	15 (65)	EASE	0.1 (210)	0.1 (420)	EASE
GRP manufacture	40 (173)	100 (433)	Industry/HSE/ Kolstad et al	60 (260)	180 (779)	Kolstad et al /HSE/EASE	1.2 (820)	8 (820)	Riskofderm /TGD

4.1.1.2 Consumer Exposure

In section 2.2 the IUCLID data on the production range for styrene is given as between 2.2 and 4.9 million tonnes annually, with imports between 30 and 150 thousand tonnes. Most of this is used in the production of polystyrene and in other polymers including acrylonitrile-butadiene rubber, unsaturated polyester and other resins. Many of these products are used in consumer goods; a table is given in section 2.3.

This section is concerned with the exposure of consumers to styrene via consumer products. Consumers may be exposed through the release of residual styrene monomer from polymeric styrene products. This exposure may follow emission into the atmosphere from materials such as carpet backing and underlay, or migration from food wrappings into food. Consumers may also be exposed to the monomer if they use styrene-containing resins for filling or repair of wood, glass fibre or metal or use styrene based adhesives. The resin exposures may be infrequent and acute but are potentially high – comparable to equivalent work in an occupational setting.

Styrene is also present in the diet from natural sources, from chewing gum and tobacco smoke. Exposure to styrene arising from natural sources and from contamination of food, water and the air following emissions from industrial plant are considered in section 4.1.1.3.. Some brands of chewing gum contain food-grace styrene-butadiene rubber, which may be a source of residual styrene monomer. Styrene in tobacco smoke is not a product of the styrene industry but a by-product of combustion. However, it can contribute significantly to total styrene uptake.

This section uses measured and modelled data to predict exposures from the above sources.

There is some information available on styrene in indoor air and this is referred to when appropriate for comparison with the model calculations. However, while there is some information on residue levels in various polymers, there is little to connect particular products with specific airborne exposures and there is little information on emission rates. Consequently there are some uncertainties in the calculations of human uptake. However, an attempt has been made to differentiate between continuous low-level exposures to airborne styrene and the short term, sporadic exposures which may arise from certain consumer activities.

The predicted exposures are in turn used to predict the uptake, in this context the amount inhaled, swallowed or on the surface of the skin. The extent of absorption following exposure, and consequently uptake, is calculated in the risk characterisation section.

4.1.1.2.1 Continuous low-level exposure arising from the use of polymeric materials releasing free monomer

Exposure from residual monomer in polymeric building materials and carpets

Low concentrations of styrene have been reported for indoor air as a result of emissions from polyester resin flooring materials (McClellan, 1994). One poorly reported study suggested that plastic floor tiles emitted most of the styrene within the first two weeks of manufacture (Gadalina *et al*, 1969). After a month the emissions appeared to fall dramatically and concentrations of styrene in the air had gone from initial levels of over 200 ug/m^3 to almost nothing.

Styrene butadiene rubber (SBR) is usually made by polymerising the two monomers in water (see section 2). Residual styrene is often found in polymer dispersions used for products such as carpet backing. Although no precise data are available, the industry has indicated that 200 ppm is the upper limit for free styrene in these polymer dispersions. A tolerance limit of 0.005 mg/m^3 for styrene emissions from carpets has been set by G.U.T., the German carpet manufacturers trade association, but this may not apply across the rest of Europe.

There is no information on other polymeric materials containing styrene, although the data presented below in relation to food contact materials may be relevant, since some of these materials have other uses in building materials. However, to make use of the information on residual monomer level, further information would be needed on the rate of emission of the styrene into the atmosphere.

Studies of indoor air quality present data on ambient airborne styrene levels in the homes or other buildings. The US TEAM study presented an average level for residential indoor air of 6.12 ug/m^3 with a median of 1.32 ug/m^3 (2125 data points). Interestingly, indoor air levels were greater than levels in ambient air, which had a median level of 0.507 ug/m^3 (McClellan, 1994) most likely due to smoking. A Canadian study of 757 single family homes quoted in the same review, presented average 24 hour styrene concentrations across all homes of 0.28 ug/m^3 ranging from not detected (<0.48 ug/m^3) to 128.93 ug/m^3. A much smaller Canadian study (7 out of 12 homes) had averages of 4 ug/m^3 in autumn (the highest value around 7 ug/m^3) and an average of 2.4 ug/m^3 (range 1 to 4 ug/m^3) in winter. No information on smoking was included in these Canadian studies.

Stepwise regression analysis of the TEAM study data suggested that personal exposures were most strongly associated with smoking, working at plastics or chemical plant, exposure to paints and building scale models. In the Canadian study, information was collected on home age and type, ventilation and heating systems, occupancy, and activities including painting and acquisition of new furniture. This information was not available in the study report quoted by McClelland (1964).

A review by an American group (Holcomb and Seabrook 1995) of recently published studies of indoor air concentrations of VOC's suggested that styrene concentrations in public places averaged 8 ug/m^3 (6 studies) and in homes averaged 1.6 ug/m^3 (5 studies), all roughly in line with the TEAM studies.

However, while these studies clearly indicate that styrene is present in indoor air and, in the case of the TEAM study, the possible importance of certain activities, particularly smoking, there are no measured data directly linking the presence of particular materials with particular styrene levels. This is expected, since there are a considerable number of variables to take into account in any study of contaminants indoor air.

Given the variability of the information obtained, it is difficult to select one value alone that represents an overall average exposure. It is also important to differentiate between continuous low-level exposure and sporadic "spikes" of exposure, which may occur when, for example, new carpets are fitted. This latter exposure is discussed in section 4.1.1.2.2. For the continuous low-level exposure from building materials and carpets, the G.U.T. tolerance limit of 000.5 mg/m^3 (5 ug/m^3) compares well with the US average of 6.12 µg/m^3 and with the results of the smaller Canadian study. This value will therefore be used as an indicator of worst case routine ambient indoor styrene levels arising from polymeric materials, including carpets.

Assuming an ambient level of exposure of 0.005 mg/m^3 and an adult inhalation rate of 11.5 l/min (CONSEXPO default) for a 24-hour exposure, the daily exposure from this source will be in the region of 80 µg/day (0.08 mg/day).

Exposures from migration of residual monomer from food contact materials.

Styrene-containing polymers used for food contact materials have been shown to contain quantities of residual monomer up to a maximum of 2500 mg/kg (0.25%) MAFF (1983). Styrene may therefore leach into food from articles made from these materials (e.g. kitchenware, cups, wrapping films, etc.) or may be actually present in food as a flavouring agent. Table 4.24 sets out the data.

Table 4.24 Levels of residual styrene in polymer and copolymer food contact materials and articles in 1980 (MAFF, 1983)

Polymer or copolymer	**Residual styrene (mg/kg)**	
	Typical	**Maximum**
Polystyrene	300-1000	2,500
Expanded polystyrene	300-1000	2,000
High impact polystyrene	300-1000	2,500
Acrylonitrile-butadiene-styrene (for food tubs)	200-300	600
Acrylonitrile-butadiene-styrene (for other uses)	300-1000	2,000
Styrene-acrylonitrite	600-1200	2,000
Methyl methacrylate-butadiene-styrene	ND-10	30
Styrene-butadiene block copolymer		
Glass reinforced plastic	20-200	1,000
Styrene-acrylic copolymers	60 in latex	
Styrene-butadiene-styrene[a]		
Styrene-soprene-styrene[a]		
Methylstyrene-vinyltoluene resin[a]		
Styrene-butadiene – raw polymer	10-30	
- cured polymer		

[a] – not used for direct food contact purposes

This figure of 0.25% for residual monomer may, however, no longer apply to current materials. The Association of Plastic Manufacturers in Europe (APME) suggest that most polystyrene is now made to have a residual content of 300-600mg/kg with typical levels being ~400 mg/kg. Expandable polystyrene is an exception, the typical level being ~800 mg/kg (see section, 3.1.0.1.4). APME states that the maximum figure is ~0.1% (1000 mg/kg) and this should only be seen in some expandable polystyrene representing a small proportion of the total. American studies (Jickells *et al* 1993) on thermoset polyester cookware showed a maximum free styrene content of 380 mg/kg (0.038%).

Measurements on levels in food indicate that migration of the monomer from plastic to food can occur. However, it is not always easy to determine the source of the styrene as styrene can occur naturally in foodstuffs uncontaminated by plastic containers. The information presented below is taken from Chapter 3 but is repeated here in the context of food contamination for consumers.

An extensive UK survey of styrene monomer levels in styrene-based plastic packaging materials and their contained foods (133) was carried out, examining a wide range of retail foods of different brand names (Gilbert and Startin, 1983). Some of the foods covered were

yoghurts, creams, salads, coleslaws, soft cheeses, margarines, hot and cold beverages from dispensing machines, spreads, fresh and cooked meats, candied fruits, fresh strawberries and take-away fast foods. Analysis of the plastic containers showed levels of monomer ranging from 16 to 1300 mg/kg, although the majority of containers (73%) had styrene levels in the range 100-500 mg/kg and only five plastic tubs had levels exceeding 1000 mg/kg. Analysis of the food contents of the plastic containers showed levels of monomer ranging from <1 µg/kg to 200 µg/kg, although the majority of foods (77%) had styrene levels below 10 µg/kg and 26% of the total number analysed had levels below 1 µg/kg.

MAFF (1983) also monitored styrene levels in food products in the UK in 1981. The mean concentrations tended to be higher in dairy products: 26 µg/kg in yoghurt, 22 µg/kg in dessert products, 16 µg/kg in soft cheese, 11 µg/kg in cream and 10 µg/kg in spreads. Lower levels were found in coleslaw, fresh meat, glace fruit, fish, strawberries and take-out foods. Levels in food containers ranged from 197 – 718 µg/kg.

Similar monitoring exercises were carried out in 1992 and 1994 (MAFF, 1994). In the most recent survey, 248 samples of food, including hot and alcoholic drinks, fruit and vegetables, meat, take away meals and a variety of dairy produce, in a variety of pack types and sizes were analysed. The majority of the samples ranged from below 1 µg/kg (the detection limit) to 60g/kg. Higher levels were found in some low fat spread samples, up to 100 g/kg, although other samples of similar materials contained less than 20 µg/kg. Milk and cream products sold in individual portions (~10 g) had styrene contents from 23-223 µg/kg, with a mean of 134 µg/kg. For other food types, mean values were less than 30 µg/kg. In general, for each food type higher levels were found for products with a higher fat content or packed in smaller containers. Overall the styrene levels were considered to be similar to those in the pervious surveys.

Miller *et al* (1994) cited a TNO literature review of surveys of styrene in foodstuffs. The highest concentrations reported were in beer (10-200 µg/kg), coffee (20-360 µg/kg), bilberries (25 µg/kg) and blackcurrants (60 µg/kg). Styrene was detected in 62 food products but was not quantified in most of these.

In a Canadian survey of a wider range of foods, samples of 34 food groups (each a composite of individual food items, combined in approximate proportion to their consumption in the Nutrition Canada Survey) were collected from retail outlets (Environment Canada, 1993). Styrene was not detected in any of the 34 food groups (detection limits 1.0 µg/1 for liquids and 0.005 µg/kg for solids).

One study measuring emission of styrene from containers into food has been found Varner and Breder (1981a) measured leaching of styrene from foam cups into water, tea and coffee of 0.0077, 0.0078 and 0.0078 µg/cm^3 respectively, with leaching into ethanol solution reported to be greater.

The studies of styrene in food include levels of residual monomer in food containers and foodstuffs. There is one study of emission rates into food from a container. The levels vary over a wide range and, given the nature of the surveys, it is not feasible to separate the contributions of natural sources, environmental contamination, food preparation and food containers. However, it is clear that residual styrene in containers can migrate into foods and that higher concentrations are found in foods containing higher levels of fat and presented in smaller pack sizes. MAFF (1983) estimated that the average and maximum likely per capita uptakes of styrene derived from the average UK diet are approximately 1 µg/day and 4 µg/day, respectively. However, while this estimation clearly made use of the survey data, the calculation refers to total styrene intake, not just the contribution from packaging.

A model has been developed for estimating the daily dietary uptake of styrene from food stored in polystyrene containers (Lickly *et al*, 1995). This model uses an amended form of the US Food and Drug Administration's consumption factor and food-type distribution factor approach. The consumption factor is defined as the fraction of an individual's diet likely to have contact with a given packaging material and for each material, food type distribution factors are defined which indicate the fraction of the food that is aqueous, acidic, alcoholic and fatty. The food distribution factors are combined with extraction data on the additive of interest, suing appropriate solvents, under the most severe temperature and time conditions to which food will be exposed while in contact with the packaging.

This approach is acknowledged to be very conservative and the amended approach takes account of the types of food in contact with polystyrene and refines the migration calculations to take more account of real conditions of use. For the average American diet, where polystyrene usage may be higher than in Europe, the contribution of styrene from polystyrene packaging to dietary intake was calculated to be 9 µg/day.

Styrene is scheduled for consideration by the EC Scientific committee for Food, as part of its routine review of substances used in food contact plastics. A survey has been conducted in the UK to aid this review (MAFF, 1999). In this survey, samples of individual foods from 20 food groups were purchased from retail outlets at five different locations in the UK, in 1997. The samples were prepared as for consumption and analysed for styrene, using an analytical technique with a detection limit of 0.3 µg/kg. A total of 100 food samples were analysed, including samples of food which had been packaged in material made from styrene (food samples in the 'oils and fats', offal', 'poultry', 'dairy products', 'carcass meat' and 'fish' groups included samples which had been packaged in styrene-containing materials). Styrene was detected in all food groups, with the exception of 'potatoes'. The highest level detected was 14 µg/kg, in a food sample from the 'oils and fats' group. Levels were generally highest in this food group as well as in the food group 'nuts' (6-12 µg/kg). Migration from packaging may have contributed to the styrene levels in 'oils and fats', but not to those in 'nuts' as these food samples had apparently not been in contact with packaging made from styrene. It is possible that where styrene was detected in food groups not packaged in material made from styrene, contact may have occurred earlier in the production chain or some contamination from environmental sources may have occurred; natural production is also a possible source of the styrene in these groups, although this is considered less likely, in view of the diversity of non-packaged foods sources in which styrene was detected (animals, vegetables and fruit).

Overall, this study indicates the presence of styrene in a variety of food types, at levels up to 14 µg/kg. The estimated daily dietary exposure to styrene can be calculated from the mean levels of styrene in each food group together with average daily consumption data for each food group. Daily consumption data for each of the 20 food groups analysed in this study have been estimated in a national food survey conducted in the UK (MAFF, 1997). Based on the levels of styrene in each food group, and daily consumption estimates for each food group, the mean daily dietary intake of styrene, per person, is estimated to be between 2.1 and ~3 µg/day (see Appendix 8), equivalent to between 0.03 and 0.04 µg/kg/day, for a 70 kg adult (MAFF, 1999). As this estimate is based on a recent and extensive study of styrene in a wide variety of food groups, the upper value of 3 µg/day (3×10^{-3} mg/day) will be used in the risk characterisation.

A worst case scenario would be to assume that an adult has a styrene intake of between 2.1 and 3 µg/day.

4.1.1.2.2 Other sources of continuous consumer exposure

Chewing gum

One study noted that styrene may be present in chewing gum made from food-grade butadiene-styrene rubbers (SBR), up to a level of about 30 mg/kg in SBR (MAFF, 1983).

Information on long-term chewing gum consumption is available from UK dietary surveys. Average consumer consumption of chewing gum for adults (aged 16-64 years) is 2 g/person/day, with a 97.5th percentile consumption of 11 g/person/day (Gregory *et al*, 1990).

An alternative scenario is to use per capita consumption of chewing gum based on average consumption. Figures collected by the European Association of the Chewing Gum Industry (EACGI, 1995) show average consumption in the EU to be less than 1g/day. Assuming the "heavy user" consumes three times this value, 3 g/day can be used in the calculation.

According to the EACGI, some brands of chewing gums contain a maximum of 2.4% food-grade butadiene-styrene rubber. If the residual level of monomer in the polymer is 30 mg/kg, then using the 97.5th percentile consumption value as a worst case, and assuming that all the available styrene is ingested, the daily uptake of styrene would be 8 µg/day (8 x 10^{-3} mg/day). Using the per capita consumption estimate, for a 'heavy user', then daily uptake is less than 3 µg (3 x 10^{-3} mg) styrene. The estimates based on the 97.5th percentile consumption data will be taken forward to the risk characterisation.

Tobacco smoke

Styrene is produced as a consequence of combustion of tobacco and therefore occurs in tobacco smoke. It is not supplied for use in tobacco. As a consequence, this source of potential exposure is not subject to consideration under EEC/793/93. However, although clearly not a product of the styrene manufacturing industry, the available information on styrene exposure from tobacco smoke helps to set the contributions from other sources into context. It is presented only for information, and exposures arising from this adventitious source will not be included in the risk characterisation.

In studies of whole smoke from cigarettes from different countries, the styrene content was found to be between 18 and 48 µg/cigarette. The US TEAM study indicated that smoking was an important contributor to styrene levels in indoor air, with concentrations of styrene in indoor air higher in homes with smokers. The concentrations so styrene in personal air samples for smokers was found to be higher and when styrene levels in breath samples were measured it was found that smokers had average levels of 1.1 µg/m^3 compared with 0.3 µg/m^3 for non-smokers (Wallace and Pellizzari, 1986). The excess overnight concentrations of styrene in air in homes with smokers, measured by the difference in weighted geometric means in homes with or without smokers averaged over the surveys was 0.53 µg/m^3 (McClellan, 1994).

Assuming an inhalation rate of 11.5 l/min (CONSEXPO default) for a 24-hour exposure, then someone sharing a house with a smoker increases their styrene uptake by approximately 9 µg over a 24 hour period. For a smoker, rounding the styrene content for cigarettes to 20 and 50 µg/cigarette means that a heavy smoker (say 20 a day) may increase their styrene uptake to between 0.4 and 1 mg/day.

While the limited measurements available clearly show a difference between smoking and non-smoking households and between the personal samples of smokers and non-smokers, the calculations of uptake, particularly in relation to the styrene content of smoke from individual cigarettes are tenuous. For comparison with other uptake values, living in the

same house as a smoker is assumed to add 9 µg/day (0.009 mg/day) to styrene uptake, whilst smoking is assumed to add 400 µg/day (0.4 mg/day) to total styrene uptake.

4.1.1.2.3 Sporadic emissions from laying of new carpets

This section attempts to quantitate the "spikes" of styrene exposure that can occur with some sporadic uses of styrene-containing materials.

Fitting of new carpets

A study is available which looked at emission of VOCs from building materials and consumer products (Wallace *et a*l, 1987). Carpet and carpet glue were among the consumer products studied. Wood panels were carpeted using carpet glue and allowed to age for one week. They were then placed in an environmental chamber, which had a measured constant air flow. Sampling took place after 12 hours equilibration and an emission rate was calculated from the equation:

$$C_{eq} - C_0 = S/Va$$

Where:

C_{eq} = the equilibrium concentration (µg/m^3)
C_0 = the background concentration
S = the source emission rate (µg/hr)
V = the chamber volume (m^3)
a = the air exchange rate (hr^{-1})

Styrene concentrations in the chamber were 8.4 µg/m^3 and the emission rate for styrene was 98 µg/hour. Levels of styrene in a new office building, sued for comparison, were also between 8 and 9 µg/m^3, although this may have included a contribution from other materials.

The US EPA has stimulated research on indoor air quality and its relevance to sick building syndrome. Carpets are one of several potential sources for volatile organic compounds (VOCs) and were recently studied for their off-gassing potential. One report (RTI, 1992), used data from chamber studies on new styrene-butadiene rubber backed carpets. Only the carpet itself, and not the associated adhesives, was investigated.

Styrene was found in emissions from all 19 products tested and the EPA model EXPOSURE was used to calculate exposure profiles from a simulated composite carpet containing styrene and the other common constituents. The predicted results showed styrene levels in the test house would rise to a peak of 90 µg/m^3 within a day but then fall rapidly to less than 10 µg/m^3 after 5 days. A worst case scenario for the composite carpet predicted styrene air levels peaking at 420 µg/m^3 within a day and falling to below 50 µg/m^3 after 5 days. When this worst case model was combined with standard time activity patterns, an exposure profile was produced. Peak exposures were predicted to occur 7 to 15 hours after installation of the carpet. Cumulative inhalation exposures (assuming a breathing rate of 1.1 m^3/hour) confirmed that the exposure peak was in the first 24 hours and was about 2 mg.

The study by Wallace *et al* (1987) does not make clear whether the carpets are new and hence whether there is a contribution from adhesive and/or carpet. However, the panels were in any case allowed to age for one week, which implies, if the EPA predictions are correct, that the major emission peak would not be covered. The EPA study does not cover adhesive emissions. Recent information obtained from TNO (NL) and industry (EPDLA)

shows that styrene is not used as a solvent in adhesives for carpets. TNO states that during the period 1990-1993 (extensive TNO study on carpet fitting and exposure to solvents), styrene was not a relevant solvent in adhesives for carpets in products on the market in the Netherlands. EPDLA (European Polymer Dispersion and Latex Association) states that to the best of their knowledge, styrene is not used as a solvent in carpet adhesives.

According to the study of Wallace *et al* (1987), for an 8-hour exposure to the concentrations found in the chamber (8.4 µg/m^3), perhaps typical of an office, the calculated level of styrene uptake is about 50 µg (for an adult breathing 11.5l/min). A 24-hour exposure more typical of some domestic exposure, would lead to an uptake of 140 µg for an adult. The RTI experiments led to model predictions that styrene levels following carpet installation peaked within the first day and then fell rapidly. The cumulative exposure over the first day was about 2 mg. This peak value will be used as the 24-hour exposure for the laying of new carpets, for an adult.

4.1.1.2.4 Styrene-containing resins

Styrene is added to unsaturated polyester resins to act as a cross-linking agent and solvent. The DIY market for these products can be split into resin pastes and liquid resins. The resin pastes are used predominantly for cars, although some can be formulated specifically for household repairs such as wood filling. Liquid resins are used mainly for boat and car repair; the resin is used in a glass reinforced laminate.

Consumers may also choose to build their own boats. A separate scenario will be described for this based on information from occupational boat building.

Resin paste

The resin pastes typically contain 11 – 18% styrene blended with inert fillers and plasticisers. Consumer kits containing polyester resin paste and hardener vary in volume between 60 and 600 ml, with the commonest size being 250 ml.

Liquid resin

Liquid resins typically contain 30 – 40% styrene. Although the exposure scenario from the small scale use of liquid resin is likely to be similar to that for polyester resin repair paste, the higher styrene content may give rise to higher exposures. Specialist outlets which also supply professional users may supply industrial pack sizes up to 40 kg. Volatilisation of styrene during the use of these kits depends on a number of factors but 10% of the total, during use, can be regarded as a worst case.

During application exposure occurs via the dermal and inhalation routes. The resins are probably applied, by hand, using a brush.

Inhalation exposure for resin kits and liquid resins

No measured data on exposure for consumer use of these products are availableThe consumer exposure during use of these products can be modelled. However, while the manufacturers recommend adequate ventilation during use of these products, an initial worst case scenario must assume that such products may be used in enclosed, poorly ventilated

spaces. The exposure consequences of such a worst case scenario may be calculated as follows, using the EC Algorithm and US EPA SCIES models. The calculations are presented in Appendix 4. Industry has also provided scenarios for consumer use of unsaturated polyester resin kits and liquid resins. These are given in Appendix 5.

Exposure prediction using the EC Algorithm method

The exposure scenario is that the product is used in a small garage and the immediate volume of air around the operator amounts to 2 m^3. No ventilation takes place and the operator stays in the concentrated atmosphere.

The EC Algorithm predicts that over a one hour period, a person working in this concentrated atmosphere will inhale approximately 29 g of styrene.

Exposure prediction using the US EPA SCIES Program

This is the US EPA computerised model for estimating the consumer exposure to chemical substances. The basic scenario remains the same but SCIES requires that some additional assumptions be made. The volume of air in which the contaminant is diluted is divided into two zones. Zone 1 represents the working area and is assumed to be a small room. However the air within this room is expected to mix to a limited extent with the rest of the building, Zone 2. One consequence of this model structure is that while the air around the working area may contain less styrene, the person coming away from the work area may still be exposed to a contaminant and for a much longer period, even though the work has been completed.

Using SCIES, the average concentration in the work zone (Zone 1) during the period of use is 4.7 g/m^3 and the peak concentration in that zone is 7.7 g/m^3. Some exposure to lower concentrations will be maintained after the period of use. The person carrying out work is predicted to inhale approximately 20 g/event. For resin pastes, the amount inhaled is reduced to approximately 8 g/event.

Industry scenarios

These are based on TGD algorithms.

(a) Reasonable "worse case" consumer use of body filler paste: (use: filling dented bodywork on a car within a closed garage)

Assumptions: Amount of product used per event, q = 450 g (based on IK median pack size of 250 mls and product density of 1.8 g/cm^3). In such applications it is difficult to use large amounts of catalysed filler at any one time as it cures before it can be used. The need for mixing, spreading, curing and sanding to take place increases the cycle time for application of each layer of paste and consequently limits the amount of paste to be used in an hour. 450g would provide 5 x 90 g mixes of catalysed resin.

Weight fraction of styrene in-product, W_f = 0.12 (based on typical styrene concentration filler paste).

Fraction of substance that volatilises during duration of exposure R = 0.05 (based on industry laboratory work on VOC emissions which indicates 4.5% of total styrene content lost from a filler containing 12% styrene during mixing with catalyst and the cure process).

Volume of air surrounding the user, V_r = 52 m^3 (based on working in a garage of volume 35 m^3 containing a vehicle of closed volume 9 m^3) implies net workspace volume of 26 m^3. Simple arithmetical treatment would imply one air change an hour would be the equivalent of a two fold increase in diluent volume. Although the air change rate would be expected to be >=2 changes/hour, 1 air change/hour has been used to compensate for periods of inhalation of higher concentrations when working close to the source of emissions.

- Ventilation rate of adult, V_{inh} = 1.3 m^3 /h (US EPA for painting work etc)
- Duration of Exposure t = 1h (likely normal maximum)

Average concentration of styrene in air, $C_{air} = q \times W_f \times R \times V_r^{-1}$

C_{air} = 450 x 0.12 x 0.05/52

C_{air} = 52 mg/m^3 (converts to ~ 12 ppm)

Amount of styrene absorbed in inhalation = $C_{air} = V_{inh}$ x fraction absorbed x time exposed

= 52 mg/m^3 x 1.3 x 1 x 1h

= 68 mg (approximately 1 mg/kg bodyweight for a 70 kg individual)

This assessment is in agreement with general experience of resin kit use, particularly in light of the expected frequency of such exposures (perhaps 1-3 hours per day).

(b) Reasonable "worse case" consumer use of liquid resin kit:

Assumptions:

- Amount of product used per event, q = 550 g (based on standard 500ml "consumer" pack size in UK)
- Weight fraction of styrene in product, W_f = 0.40 (based on typical maximum styrene concentration in liquid resin).
- Fraction of substance that volatilizes during duration of exposure, R = 0.075 (based on industry laboratory work on emissions from hand lay up).
- Volume of air surrounding the user V_f = 52 m^3 (based on working in a garage of volume 35 m^3 containing a vehicle of closed volume 9 m^3) implies net workspace volume of 26 m^3. Simple arithmetic treatment implies one air change an hour would be the equivalent of a two fold increase in diluent volume. Although the air change rate would be expected to be >=2 changes/hour, 1 air change/hour has been used to compensate for periods of inhalation of higher concentrations when working close to the source of emission.
- Ventilation rate of adult, V_{inh} = 1.3 m^3 /h (US EPA default for painting work etc)
- Duration of exposure t = 1 hour (likely normal maximum)

Average concentration of styrene in air, $C_{air} = q \times W_f \times R \times V_r^{-1}$

$C_{air} = 550 \times 0.4 \times 0.075/52$

$C_{air} = 318$ mg/m^3 (converts to ~ 75 ppm)

Amount of styrene absorbed in inhalation = $C_{air} = V_{inh}$ x fraction absorbed x time exposed

= 318 mg/m^3 x 1.3 x 1 x 1 hour

= 413 mg per event [approximately 6 mg/kg bodyweight for a 70 kg individual]

Unaccustomed exposure to the distinctive odour of styrene is likely to encourage the user to seek better ventilation or fresh air. The frequency of such exposures to those purchasing consumer kits of liquid resin is unlikely to be more than a few hours/year.

Discussion

The predictions produced by the EC Algorithm method and the US EPA SCIES Program are probably overestimates of exposure, although the degree of over estimation is difficult to determine. Firstly, the assumption that all of the weight fraction of the product is available for volatilisation may be wrong. Secondly, the models assume volatilisation in a confined space and it is expected that outdoor use would significantly reduce exposure owing to natural ventilation.

Another factor which suggests that these models may over-estimate exposure is that styrene concentrations above 1000 ppm (4.2 g/m^3) produce irritation and lacrimation sufficient to force the user to seek fresh air. However, a further calculation in Appendix 4, using twice this value as a “bearable maximum” and a starting point for predicting exposure, suggests the amount inhaled will be approximately 11 g/event.

The above predictions vary to a considerable degree, depending upon the assumptions made. However, use in poorly ventilated spaces, could mean that considerable amounts of styrene are inhaled. For the purposes of risk assessment of the use of liquid resins, the industry RWC scenario prediction of 413 mg of styrene inhaled per event will be used. For the purposes of risk assessment of resin pastes, the industry RWC scenario of 68 mg/event will be used. These values are preferred as they appear to be based on more realistic assumptions than either of the two model predictions.

Dermal exposure

The use of resin kits could give rise to dermal exposure. The EC Algorithm for dermal exposure scenario for a reasonable worst case exposure assessment assumes that skin contact will arise through accidental spillage, or failure to wear protective equipment such as gloves during application of the resin. The calculations are presented in Appendix 6.

If the typical maximum amount of styrene in a liquid resin product is 0.495 g/cm^3, the amount of styrene predicted to be on the skin surface per event is approximately 14 g. For resin pastes, which contain less styrene, the amount predicted to be on the skin surface per event is 5.5 g.

UK industry has suggested what is considers to be a more realistic worst case scenario for dermal exposure arising from the use of liquid resins. The calculations are presented in

Appendix 7. Should gross contamination occur due to significant accident spillage etc then removal of excess resin before continuing work would be expected for practical reasons, i.e. it is difficult to work with too much resin on the hand as it would stick to the workpiece.

The amount predicted to be on the skin available for uptake (as 10% evaporates) is calculated to be approximately 11 g/event. Industry also suggest that skin contamination would be unlikely when using resin paste as the product is a thick paste handled with a filling knife or plastic spreading tool included in consumer kits.

The above dermal exposure calculations for the skin surface vary depending upon the details assumed in the use scenario. Furthermore, skin contact could be considerably reduced by wearing appropriate gloves. The amount absorbed across the skin is a separate issue, considered in the risk characterisation section. However, the amount absorbed will depend upon the rate of curing of the resin (UK industry has some comments on this issue in Appendix 7), and the amount of time that the resin remains on hands before washing.

For the purposes of risk characterisation, the amount of styrene deposited on the skin available for uptake will be assumed to be 11 g/event for liquid resins (on the grounds of practicality). Although the levels on the skin for use of pastes are likely to be much lower than 5.5 g/event because of the physical nature of the product, some of the product is likely to get on to the skin, so 1.7 g/event will be used for paste kits as this is the only available value. The uncertainty of this value will be taken into account at the risk characterisation stage.

4.1.1.2.5 Boat Building

Information received from Member States, indicates that in several EU countries (including Sweden, Finland and the Netherlands) consumers do build their own boats. We have no direct information on exposures during this activity but will use information from the occupational setting to derive a scenario.

Essentially the process that consumer boat builders must go through using styrene in GRP production is the same as used in boatyards. The main difference will be in the amount of time consumers spend carrying out this work, as they are very unlikely to spend 8 hours a day over the whole year building boats. We have assumed that a consumer is unlikely to build more than one boat and so will estimate an amount of exposure per event (i.e. building one boat). It should be noted that the actual building of the boat is likely to be spread over a very variable period of time, depending on the consumer.

Information from HSE's NEDB gives an indication of how much time is spent in workshops on the lamination part of boat building and this information has been used to estimate the amount of time it would take to manufacture a 'small' boat by hand lamination. Hand lamination is the most likely process to be used by consumers as it is less dependent on technology. It is assumed that work will take place in a poorly ventilated enclosed space, such as a shed or outbuilding, although there will likely be inward leakage of fresh air due to badly fitting doors and/or windows. It is possible that this work would be done outside, which would reduce possible inhalation exposures , but this is very dependent on the weather conditions. So we have assumed that the reasonable worst case would be inside a shed.

A 'typical' small boat can be described as 4.6 m long, 1.83m beam and 0.2 – 1.2m draft, with one layer of gelcoat and 3 layers of glass fibre. This is based on a racing sailing dinghy. In the occupational setting the average time it needs for 1 person to build a small boat hull, with no deck, i.e. a rowing boat type, using hand lay up, would be 92 hours. It would take one

man 69 hours to laminate a deck on a 4.6 m boat. Therefore for a 4.6 m boat with a hull and a deck, i.e. a sailing dinghy, it would take one man 161 hours to laminate both the hull and the deck.

The inhalation exposure values from the occupational exposure assessment will be used as hand lamination in the absence of adequate ventilation does still occur in the occupational setting. These values are:

- 8-hour TWA RWC 100ppm (433 mg/m^3);
- 8-hour TWA Typical 40ppm (173 mg/m^3);
- short term RWC 180ppm (779 mg/m^3);
- short term typical 60ppm (260 mg/m^3).

Although higher values than these have been recorded in the past, these data are based on more recent studies and are more likely to reflect current formulations of resin kits. If the 8-hour TWAs are used to estimate exposure over the total time period of lamination, assuming a breathing rate of 1.25 m^3/h and that there is an average exposure over the whole time period (as is assumed in an 8-hour TWA) this gives an RWC inhalation exposure/boat of 87,141 mg/boat to build a sailing dinghy (161 hours) and a typical inhalation exposure/boat of 34,816 mg/boat. If only a rowing boat (92 hours) was being built the RWC inhalation exposure/boat would be 49,795 mg/boat and the typical inhalation exposure/boat would be 19,895 mg/boat. If it is assumed that a consumer would spend a maximum of 8 hours in a day building his own boat (either a dinghy or a rowing boat), then the RWC inhalation exposure/event is 4,330 mg/event.

The short term inhalation exposure values for consumer boat building would be the same as the occupational values given above.

We have no direct information on dermal exposures during consumer boat building but there are few dermal exposure measurements available for the GRP industry. The Riskofderm project had a scenario (3.4) which was relevant to this industry and collected data on the application (spreading) of styrene by rolling. This process would also be used by consumers making their own boats. Rolling was studied at enterprises where plastic boats for leisure activities were produced. The duration of the scenario was in general between 35 to 110 minutes (~ 2 hr) and the formulation used contained approximately 40% styrene, 60% of styrene resin (40% styrene) and a few % of the hardener ethyl methyl ketone peroxide. For both the RWC and the typical value the exposed skin surface area is assumed to be 820 cm^2. The RWC value given is 8 mg/cm^2/8 hours and the typical value is 1.2 mg/cm^2/8 hours. For a 2-hour event, the RWC dermal exposure value is 1640 mg/event.

4.1.1.2.6 Overall consumer exposure to styrene

Consumers spend a considerable amount of time indoors potentially exposed to styrene, whether continuously from certain polymeric materials, for example, or from short term, sporadic exposures which may arise from certain activities. The latter may be comparable to or higher than occupational exposures but would be of short duration. The nature of the two exposures is different, so they will be considered separately.

The predicted exposures (the amount inhaled, swallowed or on the skin, not the amount absorbed) are as follows:

Exposure from long-term low-level sources is made up of the following components:

Emissions from polymeric building materials including carpets (inhaled)	5 µg/m^3 (80 µg/day)
Food (swallowed)	3 µg/day
Chewing gum (swallowed)	8 µg/day

In addition, exposure arising from tobacco smoking are included for comparison:

Passive smoking of tobacco (inhaled)	9 µg/day
Heavy smoker (80 cigarettes/day) (inhaled)	400 µg/day

Sporadic exposures following specific events/activities are as follows:

New carpet (inhaled)	2 mg/event
Liquid resin (inhaled)	413 mg/event
Liquid resin (on the skin surface)	11,000 mg/event
Resin paste (inhaled)	68 mg/event
Resin paste (on the skin surface)	5,500 mg/event
Boat building (inhaled)	4,330 mg/event
Boat building (on the skin surface)	1640 mg/event

The uncertainty in the predictions of exposure from polymeric materials, and the variability in the predictions of exposure arising from the use of liquid resins and resin paste suggest that more information is needed to ensure that the duration and level of exposure arising in both areas is clearly understood.

Consumer exposures to styrene may be looked at in two ways; a long term but probably very low-level exposure, and sporadic and short-lived exposures relating to particular events or activities.

Long-term exposures may arise through the release of residual styrene monomer from polymeric styrene products, following emission into the atmosphere from materials such as carpet backing and underlay, or migration from food wrappings into food.

The short-term exposures may arise when new carpets are fitted, or from the use of styrene-containing resins for filling or repair of wood, glass fibre or metal or use of styrene-based adhesives. The resin exposures may be infrequent and acute but are potentially high, particularly so in the case of the liquid resins – comparable to equivalent work in an occupational setting, e.g. boat building.

Styrene is also present in chewing gum and tobacco smoke. Some brands of chewing gum contains food-grade styrene-butadiene rubber and may release residual styrene monomer. Styrene in tobacco smoke is not a product of the styrene industry but a by-product of combustion. However, it may contribute significantly to total styrene uptake and is therefore

included for information, as it may help to set other exposures into context; values for styrene exposure from tobacco smoke are not considered in the risk characterisation.

There is some information available on styrene in indoor air that suggests that background exposures are generally very low (a few µg/m^3), of the same order as the model predictions. However, there is a lack of real data on emission rates from specific polymeric materials and consequently little to connect particular products with specific airborne exposures. There is therefore some uncertainty surrounding the calculated human uptakes, which should be considered in interpreting margins of safety.

Short-term exposures arising from the use of resins are predicted by exposure models to be quite high but are critically dependent upon assumptions made in determining the use scenarios. For liquid resins in particular, the hobbyist has the potential to be exposed to very high levels of styrene in the short term. However, the variability in the predictions depending upon the assumptions made and the shortage of real data suggests that the predictions of exposure and uptake must be treated with some caution.

The uncertainty in the predictions of exposure from polymeric materials, and the variability in the predictions of exposure arising from the use of liquid resins and resin paste suggest that more information is needed to ensure that the duration and level of exposure arising in both areas is clearly understood.

4.1.1.2.7 Combined consumer exposure

There are a number of possible sources of consumer exposure to styrene monomer. For the purposes of estimating a realistic combined consumer exposure, short-term, relatively infrequent scenarios will not be taken into account, although these would dominate in any short-term scenarios and are thus considered directly in the risk characterisation. For an adult consumer, long-term exposures may arise through the release of residual styrene monomer from polymeric building materials (80 µg/day), via food (3 µg/day) and from chewing gum (8 µg/day). The combined adult exposure from these sources would result in a total exposure of about 90 µg/day (1.3 µg/kg bw/day).

4.1.1.3 Indirect exposure via the environment

Table 3.21 from the environment section has been repeated here (Table 4.24) and gives the predicted environmental exposures to styrene and the daily human doses arising from releases from production and uses, and for releases at the regional level estimated by EUSES (log Kow=3.02, BCF=74, oral absorption rate=1 and inhalation absorption rate=1).

Table 4.24 Calculated daily concentrations for indirect exposure via the environment

Scenario	Concentration (µg/kg)							Human dose[a] (mg/kg/day)
	Fish	Plant roots	Plant leaves	Meat	Milk	Air (µg/m^3)	Drinking water (µg/l)	
Production	704	10700	11,8	1.7	0.54	99	930	0.11
Processing: GP, HI-PS	14.7	13	11.5	0.33	0.11	97	1.1	0.024
EPS	278	22	5.5	016	0.051	47	1.9	0.013
ABS/SAN	175	14	3.4	0.1	0.032	29	1.2	0.009
SB rubber/latex	187	15	3.7	0.11	0.034	31	1.3	0.009
UPE resin	433	28	2.2	0.067	0.021	18	2.9	0.006
Use of: GP, HI-PS	3.8	0.014	0.014	4.5×10^{-4}	1.4×10^{-4}	0.12	0.026	3.3×10^{-5}
EPS	3.8	0.084	0.079	2.3×10^{-3}	7.3×10^{-4}	0.67	0.026	1.5×10^{-4}
ABS/SAN	3.8	0.007	0.007	2.5×10^{-4}	7.9×10^{-5}	0.06	0.026	2.0×10^{-5}
SB rubber/latex	3.8	0.028	0.027	8.2×10^{-4}	2.6×10^{-4}	0.23	0.026	5.6×10^{-5}
UPE resin use	3.8	7.5	7.0	0.20	0.064	59	0.66	0.016
Regional	3.8	0.25	0.004	1.6×10^{-4}	5×10^{-5}	0.034	0.026	1.6×10^{-5}

[a] – human dose estimate includes intake from air and drinking water as well as from biota

It should be noted that air contributes 60-70% to these modelled estimates, and oral sources 30-40%.

In addition to the calculated levels, from a recent survey conducted in the UK on samples of individual foods from 20 food groups (MAFF, 1999) the presence of styrene was measured at levels of up to 14 µg/kg. Based on the levels of styrene in each food group, and daily consumption estimates for each food group, the highest daily dietary intake of styrene, per person, is estimated to be 3 µg/day, equivalent to 0.04 µg/kg/day, for a 70 kg adult (MAFF, 1999).

For air, the measured value of 80 µg/m^3 downwind from a reinforced plastics processing site is chosen (section 3.1.2.1). For water, the measured value of 10 µg/l is chosen as one which very few measurements exceed.

Using these concentrations, a daily intake of 0.058 mg/kg bw/day from food, air and water is estimated. It should be noted that contribution from air to this measured estimate is very high.

However, based on the modelled data, it can be seen that the daily human intake via the environment based upon typical human consumption and inhalation rates at the regional level is 1.6×10^{-5} mg/kg/day and the highest local exposure (styrene production) is 0.11 mg/kg/day. These two figures will be taken forward into the risk characterisation, as well as the value of 0.058 mg/kg/day based on measured data.

4.1.1.4 Combined exposure

For combined exposure, considerations should be given to a consumer, who is also potentially exposed via the environment. The most appropriate consumer exposures to consider are those giving rise to long-term, repeated exposure i.e. exposures from the release of residual styrene monomer from polymeric styrene products, from food and from chewing gum. These combined consumer exposures result in a daily intake of about 90 µg/day, or 1.3 µg/kg bw/day (0.0013 mg/kg bw/day) for a 70 kg adult. Based on modelled data, the highest exposure via the environment would be to someone living in the vicinity of a styrene production plant, with an estimated daily intake of 0.11 mg/kg/day. The resultant combined exposure would be dominated by the environmental exposure of 0.11 mg/kg/day. If measured environmental exposure data are considered, the daily intake via environmental sources excluding food is estimated to be 0.018 mg/kg/day; this would give a combined consumer and environmental intake estimate of 0.019 mg/kg/day. Both these values, 0.11 mg/kg/day and 0.019 mg/kg/day will be taken forward to the risk characterisation.

4.1.2 Effects assessment: Hazard identification and dose (concentration) – response (effect) assessment

4.1.2.1 Toxico-kinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

Absorption

On inhalation exposure, styrene vapour is rapidly absorbed through the lungs resulting in plateau blood levels within 3-5 hours in the rat (Withey, 1978; Plotnick and Withey, 1979; Ramsey and Young, 1978; Young *et al*, 1979; Withey and Collins, 1979). The plateau levels increased in a linear manner with increasing styrene exposure up to about 200 ppm, but the relationship became non-linear at 600 ppm, probably due to a saturation of the metabolising systems working to remove styrene from the blood (Plotnick and Weigel, 1979).

A recent study conducted on the surgically isolated upper respiratory tract of anesthetised Sprague-Dawley rats and CD mice exposed to styrene for 45 min, indicated that the percentage uptake in this region decreased (from 24% to 10% of the administered dose in rats and from 42% to 10% of the administered dose in mice) as the atmospheric concentration of styrene increased from 5 to 200 ppm (Morris, 1999 and Morris, 2000). Inhibition of cytochrome P450 by metyrapone pretreatment of the animals reduced uptake, indicating that styrene metabolism in the upper respiratory tract enhances the rate of uptake of styrene at this site in rodents.

Rates of removal of styrene from the atmosphere were measured in rats and mice using a closed chamber technique (Filser *et al*, 1993). Animals were exposed to relatively high initial vapour concentrations, estimated to be from 550-5000 ppm. From the results, taking into account information from animals pre-treated for 5 days with 150 or 500 ppm styrene, the rate of removal of styrene from the chamber at steady state increased linearly with concentrations up to about 300 ppm. Above about 300 ppm, metabolic capacity limited removal of styrene from the chamber; saturation of metabolism was observed at approximately 700 ppm in rats and 800 ppm in mice. The maximum rate of metabolism, per kg bodyweight, was approximately 3 times higher in mice than in rats. There was no significant effect on kinetics when animals had been previously treated by exposure for 6 hours/day for 5 days to 150 or 500 ppm styrene. Administration of diethyldithiocarbamate, a

specific cytochrome P450 monooxygenase inhibitor, resulted in a considerable slowing of the rate of removal of styrene from the chamber.

Styrene is well absorbed when administered orally in aqueous suspension or as a solution in corn oil, 90% of the administered dose being absorbed with eight hours in the rat (Withey, 1978; Plotnick and Withey, 1979).

It can be predicted from the lipid solubility of styrene that some dermal absorption will occur. There is one study in which whole-body absorption of styrene vapour was measured (McDougal *et al*, 1990). The rats breathed air through a mask during exposure. The fur was closely clipped and blood styrene concentrations were analysed at 0-4 hours after the start of exposure to 3000 ppm. The styrene blood concentration had not reached steady state at 4 hours. Dermal absorption was estimated to be 9.4% of the total amount absorbed when the vapour was inhaled. Absorption of liquid styrene was measured across excised rat skin *in vitro* (Tsuruta, 1982). The rate was approximately 30 μg/cm^2/hr, about half that of toluene and 5 times that of xylene. There are no studies that have measured the uptake of liquid styrene across the skin of experimental animals in *vivo*.

Distribution and elimination

Following inhalation exposure, styrene and/or its metabolites were found predominantly in adipose tissue but also in a range of organs and tissues including kidney, liver, lungs, brain, testes, heart and bone marrow of rat and mouse by tissue analysis and whole-body autoradiography (Withey, 1978; Plotnick and Weigel, 1979; Young *et al*, 1979; Withey and Collins, 1977; Bergman, 1979; Savolainen and Vainio, 1977 ;Savolainen and Pfaffli, 1978; Boogaard *et al*, 2000; Booth, 1999). A study in which the distribution of radioactivity was investigated following intraperitoneal administration of ^{14}C-styrene to groups of 5 male mice reported that at 2 hours post-dose, the highest concentrations of radioactivity were found in the adipose tissue, pancreas, liver and brain (Lof *et al*, 1983). Significant uptake of styrene into the nasal mucosa, particularly the olfactory region, has also been noted in rodents inhaling styrene (Boogaard *et al*, 2000; Booth 1999).

Campo *et al* (1999) studied the distribution of styrene in rats following inhalation exposure particularly in relation to ototoxicity (see later sections). Groups of eight male Long-Evans rats were exposed whole-body to either 0 or 1750 ppm styrene for six hours on one day, followed by a four hour exposure on the next day. Immediately after the second exposure the animals were killed and samples of the following taken: cerebrospinal fluid, whole blood, brain, auditory nerves, inner ear fluids and cochleae. Styrene was measured either by gas chromotography (GC) either directly (in fluids apart from blood) or following extraction. No styrene was detected above the GC detection limit in control animals or in the cerebrospinal or inner ear fluids. Styrene was found to be present in the organ of Corti in seven of the treated rates but was not quantifiable. Measurable levels of styrene were found in the brain, auditory nerve and whole blood, with the greatest amount found in the brain.

In pregnant mice receiving styrene by intravenous injection, styrene and/or metabolites were demonstrated to cross the placenta to the fetus (Kishi *et al*, 1989).

Styrene is eliminated from the blood in a biphasic pattern. The first phase results in a rapid decrease in blood levels (half-life values [t½] in the range 2.5-17 minutes having been reported) followed by a slower second phase (t½ values in the range 0.5-6 hours being reported) (Withey, 1978; Young *et al*, 1979; Withey and Collins, 1977; Savolainen and Vainio, 1977; Withey, 1976). In one particular study, groups of rats were exposed to high concentrations of styrene (in the range 500-1000 ppm) for 4 hours (Teramoto and Horiguchi, 1979 and 1981). Styrene concentrations were analysed in blood, liver, kidney, spleen, brain,

muscle and adipose tissue for up to 48 hours. Half-life values for the second, slower phase were calculated: the half-life (t½) in blood was 2.4 hours and similar values were found for other tissues except adipose, which was longer at 6.3 hours.

Only a small percentage (0-3%) of absorbed styrene was excreted unchanged via the breath following inhalation, oral ingestion or subcutaneous dosing. The great majority of styrene taken up by the body was converted, mainly in the liver, to metabolites which were excreted in the urine; between 70 and 90% of the administered dose was excreted as metabolites within 24 hours, either in the free form or as conjugates (Plotnick and Weigel, 1979; Young *et al*, 1979; Ramsey and Young, 1978; Danishefsky and Willhite, 1954).

In a comparative study, groups of F344 rats, $B6C3F_1$ mice and CD-1 mice were exposed whole-body to 250 ppm unlabelled styrene for 6 hours/day for 0,2 or 4 days (Sumner *et al*, 1997). Following this, animals were exposed nose-only to 250 ppm [7^{-14}C]styrene for 6 hours. Urine, faeces and expired air were collected from animals for 48 hours after the last exposure and levels of radioactivity within each determined. Animals were then sacrificed and the levels of radioactivity analysed in the carcass, pelt and tissues. Urine was collected and analysed to enable the time course of radioactivity excretion to be investigated. Most of the radioactivity was excreted in the urine, within 24 hours of exposure, with some quantitative variations between species and strains. Radioactivity in the faeces accounted for 18% of the total recovered radioactivity in the rat, 4% in $B6C3F_1$ mice and 6% in CD-1 mice.

The *in vivo* distribution and covalent binding of styrene in rat and mouse nasal and lung tissues has been investigated in a series of studies (Green, 1999c; Foster, 1999a; Green, 2000a also Green *et al*, 2001a and Green *et al*, 2001b). Groups of male Sprague-Dawley CD rats and male CD-1 mice were exposed whole-body to 160 ppm ^{14}C-styrene vapour (98% purity) for 6 hours. Groups of 2 rats and 4 mice were sacrificed at 5 minutes, 1 hour, 4 hours and 24 hours post-exposure for nasal tissue analysis. Additional groups of 10 rats and 20 mice were sacrificed at the same time points for lung tissue analysis. Olfactory, nasal and lung tissues were dissected from these animals. The distribution of radioactivity in the nasal and lung tissues was determined by histoautoradiography at each time point. Whole body autoradiograms were also prepared for one animal per species at each time point. The nasal and lung tissue samples were subsequently processed and analysed by high performance liquid chromatography (HPLC) analysis and scintillation counting to measure total radioactivity, volatile radioactivity (unchanged styrene) and covalently bound radioactivity.

Over the 24-hour post-exposure period, radioactivity was particularly concentrated at the surface of the olfactory and respiratory nasal epithelial mucosa and within the olfactory mucosa and Bowman's glands; and in the lungs, in bronchiola mucosa and in the walls and lumen of the pulmonary blood vessels. In relation to the bronchiolar mucosa, radioactivity persisted for longer in the mouse than the rat. A comparison of results of 'total radioactivity' and 'non-volatile radioactivity' for both rat and mouse for nasal and lungs tissues showed there to be little difference between the distribution and content of the radioactivity present. This suggested that the majority of the radiolabel was present as non-volatile metabolites of styrene rather than unchanged styrene itself.

The majority of the radioactivity in the olfactory and respiratory nasal tissue and in the lung tissue of rats and mice was present as water-soluble metabolites which were rapidly cleared from the tissues. The concentration of these metabolites was similar in the olfactory tissue of each species. The levels of covalently-bound radioactivity in the tissues of both species were lower but remained constant throughout the study duration. The level of covalently bound radioactivity in the mouse olfactory and respiratory tissues was 3-5 fold higher, and in the lung 10-fold higher than in the equivalent rat tissues.

Groups of 6-7 male NMRI mice were exposed, whole-body, to 0, 750, or 1500 mg/m^3 styrene vapour 6 hours/day for 1, 3, 7 or 21 days (Vodicka *et al*, 2001). Animals were killed shortly after exposure on the last day of each exposure interval and fat and blood samples were taken for analysis of styrene using gas chromatography.

Blood styrene concentrations ranged from 0.99 mg/l after 1 day amongst animals exposed to 750 mg/m^3 to 2.04 mg/l at 21 days, and at 1500 mg/m^3 were around 2.48 mg/l after 1 day and 7.44 mg/l at 21 days. At the end of the exposure on day 1, blood styrene levels decreased by around 13-fold within 30 minutes. On day 21, blood styrene levels decreased by around 36-fold within 18 hours of the end of the exposure. Styrene was measured in fat but there were no differences in the levels found amongst animals exposed to 750 and 1500 mg/m^3 for 7 and 21 days. There was no information on the release rate of styrene from fatty tissue. These results indicate rapid elimination of styrene from blood in mice following either single inhalation exposure or repeated exposure.

Metabolism in vivo

The general metabolic profile of styrene in experimental animal species is illustrated in Figure 1. The epoxide styrene-7,8-oxide has been identified as a primary metabolite in rats (Fischer and Sprague Dawley strains) and mice (NMRI, DBA2, Swiss and B6C3F$_1$ strains) following inhalation or intraperitoneal exposure (Lof *et al*, 1984; Kessler *et al*, 1992; Morgan *et al* 1993c; Pantarotto *et al*, 1978). This metabolite comes from an initial conversion by enzymes of the mixed cytochrome P450 function oxidase system (see Figure 1). In a study using knock-out mice, there were no substantial differences between 2E1 null mice (ie lacking 2E1) and wild-type mice in urinary metabolites after exposure to styrene (Ghanayem et al., 2000), indicating that CYP2E1 may not be a major isozyme involved in the in vivo metabolism of styrene to SO in mice. Subsequent action by the enzyme epoxide hydrolase results in the formation of the diol, phenylglycol, which is further metabolised to mandelic acid and eventually phenylglyoxylic acid or benzoic acid, which is found as its glycine conjugate hippuric acid in many species including rats, mice, rabbits and guinea-pigs (Withey and Collins, 1977; Danishefsky and Willhite, 1954; Leibman, 1975; Ryan, 1976; James and White, 1967). The same urinary metabolites and also two mercapturic acid derivatives were identified after inhalation exposure to 25-200 ppm in the rat (Truchon *et al*, 1990), indicating that inactivation of styrene oxide by glutathione S-transferase-catalysed conjugation with gutathione (GSH) is also a significant pathway.

Other metabolic pathways are also involved in the metabolism of styrene. One of these involves ring hydroxylation to yield 4-vinylphenol (4-VP). Bakke and Scheline (1970) identified 4-VP in the hydrolysed urine of rats dosed orally with styrene, but it amounted to only 0.1% of the administered dose. Pantarotto et al. (1978) identified small amounts of 4-VP in the urine of rats administered styrene intraperitoneally. Watabe et al. (1984) reported the formation of 4-VP using ^{14}C-labelled styrene and a rat hepatic microsomal preparation. Carlson et al. (2001) found that 4-VP is a transient metabolite of styrene since it was rapidly metabolised by mouse and rat hepatic and pulmonary microsomes involving CYP2E1 and CYP2F2.

Finally, other minor metabolic pathways of styrene lead to the formation of phenylacetaldehyde (PA) and phenylacetic acid (PAA) (via side-chain β-oxidation and hydroxylation), to phenylethanol and acetophenone (via side-chain α-oxidation and hydroxylation) and to products of ring opening (Sumner *et al*., 1995). These metabolites are excreted in the urine. There are studies which have demonstrated that P450 enzymes are also involved in both the side-chain and ring oxidation of styrene.

In a study by Delbressine *et al* (1981), it was reported that following intraperitoneal administration of styrene (250 mg/kg), Wistar rats excreted a mixture of 2-hydroxymercapturic acid diastereoisomers, N-acetyl-S-(1-phenyl-2-hydroxyethyl) cysteine and N-acetyl-S-(2-phenyl-2hydroxyethyl) in the urine over 24 hours. Equivalent findings were also observed in a similar study conducted in male $B6C3F_1$ mice administered 400 mg/kg styrene via the intraperitoneal route (Linhart *et al*, 2000). The fractions of mercapturic acids isolated from the mouse urine amounted to 12-15% of the administered dose.

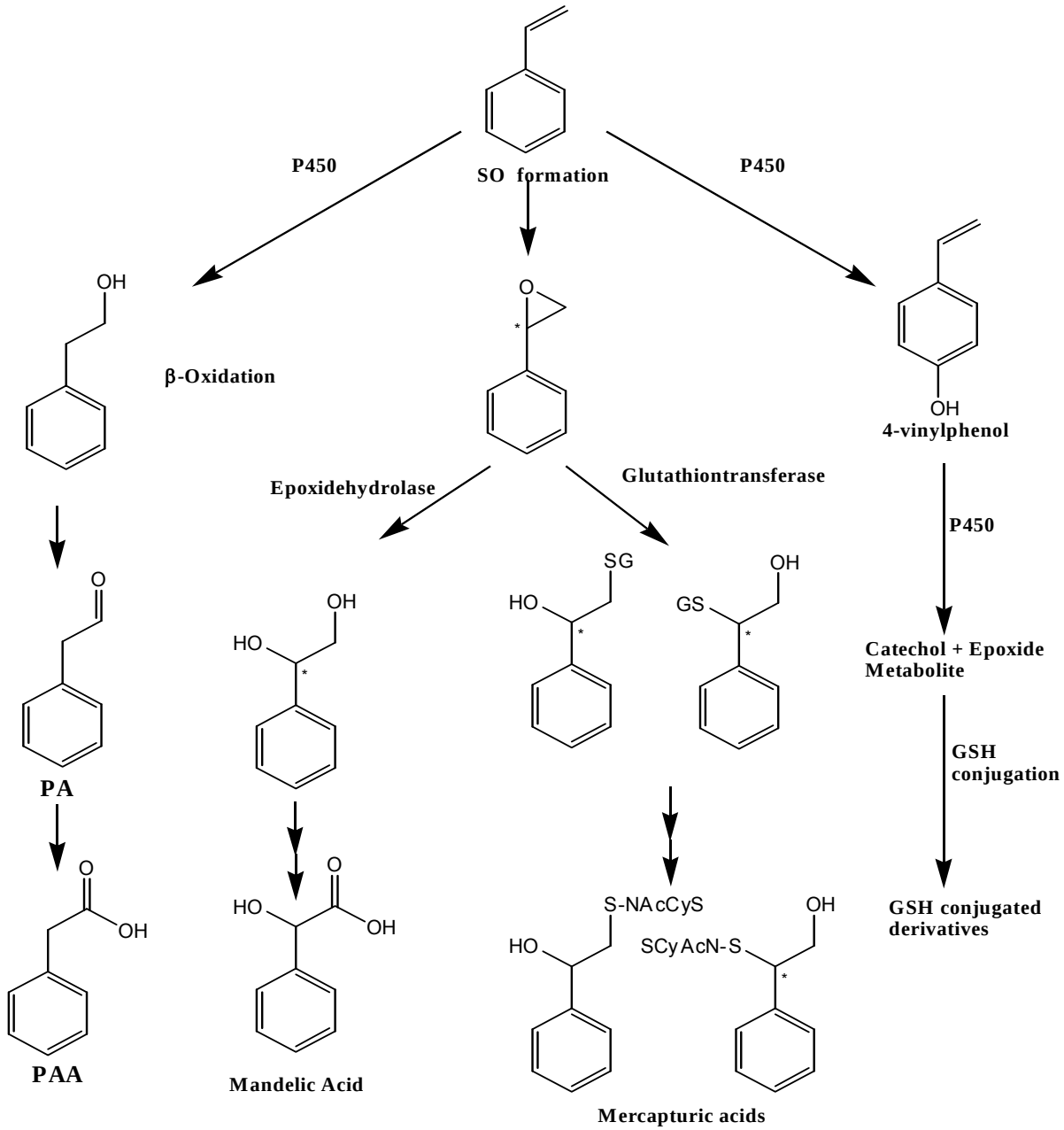

Figure 1: The metabolic profile of styrene in experimental animals

Styrene 7,8-oxide was measured in the blood of groups of rats and mice exposed to styrene, 3 hours after chamber concentrations had reached steady state values of 0-800 ppm (Kessler *et al*, 1992). This metabolite reached a maximum of about 0.3 mg/ml at around 350 ppm in the rat whereas concentrations of styrene oxide in the mouse increased from about 0.2 mg/ml at 200 ppm to 5.5 mg/ml at 800 ppm. A similar trend in mice was found in a study looking at blood styrene oxide levels after 6-hour exposure to 250 or 500 ppm (Morgan *et al*, 1993b). Therefore, there is a species difference in metabolic capacity, with mice exhibiting a substantially greater capacity than rats to produce styrene oxide at higher concentrations of styrene.

4.1.2.1.2 Studies in humans

General toxicokinetic profile following inhalation exposure

In a human volunteer study 4 healthy male volunteers were exposed via nose and mouth to 50 ppm [^{13}C8]-styrene vapour for 2 hours, whilst undergoing light physical (50W) exercise (Sumner and Greenlee 1999, unpublished; also Johanson *et al,* 2000). Expired air was collected every 30 minutes during exposure and on 8 occasions following exposure and analysed for the concentration of styrene present. Capillary blood samples were collected at 25 specified time points during and post-exposure and analysed for styrene concentration. Venous blood samples were collected prior to exposure, immediately post-exposure (2 hours) and then at 4, 6 and 23 hours and analysed for styrene oxide levels. Urine samples were collected post-exposure at the nominal time points of 0, 2, 4, 6, 9, 13, 22, 24, 42 and 51 hours throughout the study duration. Urine samples were analysed by HPLC and nuclear magnetic resonance (NMR) spectroscopy for the presence of styrene-derived metabolites.

The 4 subjects were found to have exhaled only 1-2% of the styrene dose received. Styrene concentrations in capillary blood steadily increased during the 2 hours of exposure and then rapidly decreased post-exposure. Styrene oxide was not detectable in pre-exposure venous blood.The highest concentration detected in blood was 0.0008mg/ml, seen immediately post-exposure (at 2 hours); this declined at 4 and 6 hours post-exposure (0.00044 and 0.00018 mg/ml respectively) and was not detectable at 23 hours post-exposure. In the urine, very little unchanged styrene was present. The urinary metabolites detected were mandelic, phenylglyoxylic, phenylaceturic and hippuric acids.

The uptake of styrene via inhalation was also investigated in male volunteers exposed to styrene during rest and exercise by Engstrom *et al* (1978a). Seven healthy male volunteers, aged from 22 to 30 years were exposed using a breathing valve and mouth piece to 50 ppm styrene vapour, initially for 30 minutes whilst at rest and then for 3 x 30 minutes periods of exercise with increasing workloads of 50, 100 and 150W. Exposure was interrupted for 20 minutes between the rest period and the start of the exercise periods. The mean amount of styrene expired in the first 4 hours post-exposure was measured in expired air collected continuously for the first 20 minutes with a Douglas Bag technique and then every 5 minutes via a respirometer. Styrene levels in expired air were then measured periodically for the next 15 hours. Styrene levels in arterial and peripheral venous blood samples were measured periodically in samples taken from 3 individuals during exposure and for the first 4 hours post-exposure. Needle biopsies of subcutaneous adipose tissue, from the upper lateral gluteal quadrant, were taken from all individuals prior to exposure and at 0.5, 2, 4 and 20-24 hours post-exposure, and again from 4 individuals at 1-2 weeks post-exposure. The concentration of styrene in the adipose tissue was determined by gas chromatography.

The mean uptake of styrene from inspired air across all 4 exposure periods was 63% (59-70%) and the amount of styrene eliminated via expiration was only about 2% of the amount absorbed. During the final phase of exercise (150W) uptake was 5-6 times higher than that measured during the rest period. Levels of styrene in arterial and venous blood increased

with increasing work loads. Mean levels of styrene in adipose tissue increased over the first 2 hours post-exposure, plateaued at 4 and 24 hours post-exposure, and gradually declined to negligible levels over the next 13 days.

Analysis of the data on an individual basis and with regards to individual body fat levels demonstrated that a clear relationship existed between increasing amounts of body fat and increasing levels of styrene. From the data available a half-life of styrene in human adipose tissue of 2 to 4 days was estimated (with t½ increasing with increasing individual body fat levels).

In another study by the same group of workers the uptake of styrene via inhalation was investigated in 3 male workers occupationally exposed to styrene during the processing of polyester tanks (Engstrom *et al*, 1978b). Two had been employed for several years but the third for only 2 weeks. The amount of body fat in each of the subjects was determined by means of skeletal measurements. Ambient air concentrations of styrene were measured in the breathing zone of each individual and an 8-hour TWA exposure estimated. During the same time expired air samples were collected and the styrene concentration measured. This information was then used to determine uptakes. Needle biopsies of subcutaneous adipose tissue were taken from each individual prior to exposure and on days 1, 3 and 5 post-shift. The concentration of styrene in the adipose tissue was determined by gas chromatographic techniques.

The estimated 8-hour TWA exposures were 65, 85 and 32 ppm for subjects 1, 2 and 3 respectively. Estimated mean percentage daily uptakes of styrene were 60 – 62%. Adipose tissue levels of styrene in each individual increased over the course of the 5 days of the study. From the above data the authors calculated a half-life of styrene in adipose tissue of between 2.8 and 5.2 days, depending on individual body fat content.

In earlier studies it has been shown that 59-88% of the total amount of styrene breathed in is absorbed following inhalation of styrene at atmospheric concentrations in the range 10-200 ppm in human volunteers (Fiserova-Bergerova and Teisinger, 1965; Bardodej and Bardodejova, 1970; Fernandez and Caperos, 1977; Kjellberg *et al*, 1979; Teramoto and Horiguchi, 1979; Wigaeus *et al*, 1983 and 1984; Norstrom *et al*, 1992). Continuous atmospheric exposure resulted in a rapid rise in blood styrene levels which eventually reached a plateau.

Further studies have shown that concentrations of styrene in the blood of workers increased in a linear manner with atmospheric 8-hour TWA styrene exposures of up to about 150 ppm (Brugnone *et al*, 1993 and Bartolucci *et al*, 1986). Dose-dependent kinetics have been observed in 2 volunteers exposed to styrene concentrations of 26, 77, 201 and 386 ppm for 2 hours (Lof and Johanson, 1993). Saturation of metabolism was indicated by the nature of the increases in blood styrene with increasing styrene exposure concentration. Simulations of 8-hour exposures to styrene using a physiologically-based pharmacokinetic model suggested that partial saturation began at about 200 ppm.

Elimination of styrene from the blood has been shown to follow a linear 2-compartment model, similar to that found in rats, with a half-life for the first phase being 30 minutes and for the slower second phase about 13 hours (Ramsey *et al*, 1980). These half-lives indicate that elimination takes slightly longer in humans than in rats. In workers previously exposed to styrene, the apparent blood clearance of styrene was significantly higher (2.0 l/hr/kg) than that in 6 volunteers with no previous styrene exposure (1.5 l/hr/kg) during a 2-hour experimental exposure to 70 ppm (Wigaeus *et al*, 1984 and Lof *et al*, 1986a). This observation presents some evidence that long-term exposure might increase the capacity to metabolise styrene, presumably because of enzyme induction. Uptake of styrene into subcutaneous fat was fairly rapid with plateau levels being maintained for long periods following the exposure. The t½ for elimination of styrene from the fat was calculated to be in

the range of 2-4 days, indicating a slow release, following uptake into the tissue (Kjellbert *et al*, 1979 and Engstrom, 1978a).

Styrene levels were measured in 25 samples of subcutaneous fat from workers in a styrene polymerisation plant in USA (Wolff *et al*, 1977 and Wolff *et al*, 1978b). Samples of subcutaneous fat were obtained from the buttocks by needle aspiration. Styrene was found in 13 of 17 workers who had been significantly exposed (5 ppm) within the last 3 days (5 of these not having been exposed for 2-3 days prior to sampling). Styrene was not detectable in the samples of 5 workers who had been removed from similar exposures 4-90 days previously or in 2 workers removed from "low" exposures (0.5 ppm) 2-3 days previously.

Studies exploring dermal uptake

Dermal absorption has been measured after one hand of each of several volunteers was dipped into liquid styrene for 10-30 minutes (Berode *et al*, 1985). The mean rate of absorption was 60 µg.cm^{-2}.hr^{-1}. Unfortunately, the protocol used does not facilitate the calculation of the percentage of the dose absorbed.

In a study by Riihimaki and Pfaffli (1978) dermal absorption of styrene vapour at levels of 600 ppm resulted in styrene appearing in the blood at levels which reached a plateau within two hours. The rate of absorption was calculated to be 1 µg.cm^{-2}.hr^{-1}. This low rate of absorption indicates that uptake of vapour through the skin is relatively insignificant compared to uptake via inhalation.

It was calculated from the results of a similar volunteer study (exposure to 310-762 ppm) that dermal absorption of styrene vapour contributed approximately 5% to the total amount absorbed via the lungs and skin combined (Wieczorek, 1985).

An investigation was carried out in which 8 workers in the reinforced plastics industry were monitored for levels of styrene in blood and for urinary metabolites of styrene after lamination work involving exposure to styrene (Brookes *et al*, 1980). The effects of using no dermal protection and of wearing gloves impermeable to styrene were compared. There was no significant difference in levels of styrene in blood or metabolites in urine between the shift when gloves were worn and the shift when no dermal protection was worn. Therefore, it was concluded that uptake via the skin contributed only a small amount to the total body burden of styrene during lamination work in the GRP industry.

The potential for dermal absorption of styrene has been compared with inhalation absorption in a field study of 4 workers in the fibreglass-reinforced polyester industry (Limasset *et al*, 1999). The levels of urinary metabolites of styrene (mandelic and phenylglyoxylic acid) were measured and compared for the 4 workers in the study. Each worker was assessed prior to and after work. Each of the 4 workers carried out the same job functions, in the same area of the factory for the same timeperiod (4 x 2 hours) on 4 consecutive days. However, each worker wore different protective equipment (total protection, respiratory protection only, dermal protection only or no protection). The concentrations of styrene in the working environment and within the insulator suit of the total protection were also measured.

The levels of styrene in the working environment for all workers ranged between 42-59 ppm for all of the 2-hour assessment periods over the 4 days of the study. In those individuals wearing the insulation suit styrene levels inside the suits ranged between 0.05-3 ppm for all of the 2-hour assessment periods. Urinary metabolite levels post-shift were significantly increased in samples obtained from the workers who did not wear respiratory protection (no protection or dermal protection only) compared with pre-shift levels. Levels of urinary metabolites in workers with respiratory protection but no dermal protection did not differ post-shift to levels measured pre-shift. The pattern of urinary metabolites in workers wearing total

protection was comparable with that in workers wearing respiratory protection only. Overall, the data again indicate that the major route of styrene uptake in workers in the polyester industry is via inhalation and that compared to this, absorption via the dermal route is negligible.

The *in vitro* dermal penetration of styrene was investigated in an OECD-, GLP-compliant study, using a 100% ^{14}C radiolabelled styrene and a styrenated resin formulation containing 42.7% of ^{14}C radiolabelled styrene (Gedik and Roper, unpublished, 2003).

Split-thickness skin membranes (200-400μm) prepared from fresh human samples obtained from three donors (aged 26-57 yrs old) were assessed for barrier integrity and any failing this test were rejected. In a flow through apparatus with a tissue culture medium (containing 4:1, bovine serum albumin: glucose, streptomycin and penicillin G mixture) as the receptor fluid, styrene-alone and the styrenated resin were applied to the stratum corneum of the skin samples (7 samples/donor). An activated charcoal filter was placed in the donor chamber of the apparatus to collect evaporated material from the skin surface. Post-application, the receptor fluid samples were taken hourly for 6 hours and two hourly thereafter for a further 18 hours. At the end of the 24-hour test period, the exposed skin samples were washed (skin wash) and the stratum corneum removed via tape stripping. The test material associated with the stratum corneum removed by the tape stripping was not considered by the study authors to cross the skin. The filter, the tape strips and the skin wash were assessed for residual radioactivity. For styrene, 83.7%, 0.23% and 1.13% residual radioactivity were recovered in the filter, skin wash and stratum corneum respectively. The total recovery of the applied radioactivity was 88% for styrene-alone and 95% for styrenated resin. The dermally delivered dose (receptor fluid + receptor rinse + material found in the epidermis) was found to be 1.68% and 1.23% of the applied dose, while the absorbed dose (receptor fluid+receptor rinse) was 1.25% and 1.15% for styrene-alone and the styrenated resin respectively.

Overall, it can be concluded that consistent with its chemical property, most of the styrene volatilised following dermal application and that its dermal penetration was minimal. A worst-case of 1.7% was estimated by this study for the absorption of liquid styrene through human skin. A value of 2% will be taken forward to the risk characterisation.

Metabolism and elimination pathways in humans

In inhalation studies in humans, only small amounts (about 2-3%) of absorbed styrene were excreted unchanged in the breath (Ramsey and Young, 1978; Bergman, 1979; Bardodej and Bardodejova, 1970; Fernandez and Caperos, 1977; Ramsey *et al*, 1980; Riihimaki and Pfaffli, 1978; Guilleman and Bauer, 1979). The majority (about 90%) of the absorbed material was metabolised and excreted in the urine. The major metabolic pathways are shown in Figure 1, with mandelic acid and phenylglyoxylic acid being the predominant metabolites (Caperos *et al*, 1979; Drummond *et al*, 1989; Korn *et al*, 1987; Phillippe *et al*, 1971). In contrast to experimental animals, only very small quantities of hippuric acid are produced in humans due to the low efficiency of the decarboxylation of phenylglyoxylic acid to benzoic acid. The thioethers N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine and N-acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine have also been detected in the urine of styrene-exposed workers (Truchon *et al*, 1998), indicating a small contribution to the overall metabolism of styrene from glutathione S-trasferase-catalysed conjugation of styrene oxide with GSH. More recently, Manini et al. (2003) have also detected the glucuronide and the sulphate conjugates of 4-vinylphenol in the urine of styrene-exposed workers and in volunteers exposed to approximately 12 ppm styrene (50 mg/m^3) for 8 hours. These conjugates were shown to represent about 0.5-1% of the total urinary excretion of styrene metabolites, to be eliminated with a monophasic kinetic (the glucuronide being excreted faster than the sulphate), and to significantly correlate with airborne styrene. These findings

indicate that this minor metabolic route involving the oxidation of the arene moiety of styrene also occurs in humans.

In relation to styrene oxide (SO) production, concentrations of 0.0009-0.0041 mg/ml were measured in the blood of 13 workers exposed to airborne TWA styrene concentrations of 10-73 ppm (from personal sampling measurements carried out at the end of each exposure period) (Korn *et al*, 1994). A linear correlation was established between the SO levels in blood and styrene in ambient air; estimation from this slope gave a blood SO concentration of 0.001mg/ml at an exposure level of 20 ppm styrene. This level is 10-20 times lower than that observed in rats and mice exposed to the same level of styrene (Kessler et al., 1992). In another study, styrene-7,8-oxide concentrations measured in blood samples were rarely above the detection limit of 0.0024 mg/ml in 10 workers exposed to an average of 24 ppm (sampling was conducted before and at the end of the workday) for an unstated reference period (Lof *et al*, 1986b). In addition to metabolism via cytochrome P450, *in vitro* studies are available which demonstrate that styrene can be metabolised to styrene oxide by oxyhaemaglobin present in human erythrocytes (Belvedere and Tursi 1981 and Tursi *et al*, 1983). There is also evidence from genotoxicity studies *in vitro* that human erythrocytes can metabolically activate styrene to a reactive metabolite (Linnainmaa *et al*, 1978; Norppa *et al*, 1983b; Norppa and Vainio, 1983; Norppa and Tursi, 1984; and Chakrabarti *et al*, 1993).

Between approximately 60 and 80% of the absorbed dose of styrene is ultimately converted to mandelic acid and about 30% to phenylglyoxylic acid. It has been shown that most of the mandelic acid and phenylglyoxylic acid is then excreted via the urine within 24 hours following inhalation. The excretion of urinary mandelic acid is biphasic, with a t½ for the first phase being about 4 hours and for the second phase being about 25 hours. Phenylglyoxylic acid, however, appears to have a monophasic excretion pattern with a t½ of about 10.5 hours. It has been claimed by a number studies that the excretion rates (t½ values) of these urinary metabolites are, to a certain extent, dependent upon the degree of exposure (e.g. Engstrom *et al*, 1976). This has not, however, been confirmed by other studies (Guilleman and Bauer, 1978) and the differences observed by the former studies, although statistically significant, were not large. Since the excretion rate of mandelic acid is biphasic and changes with time whereas that of phenylglyoxylic acid remains constant, the ratio of mandelic acid/phenylglyoxylic acid is variable, ranging from about 3-4 immediately after exposure to about 0.8-1 at 24 hours after exposure (Guilleman and Bauer, 1979; Guilleman and Bauer, 1978; Brookes *et al*, 1980).

In terms of bioaccumulation potential, there is a relatively short half-life of styrene in blood but a relatively long half-life in adipose tissue; therefore it is reasonable to conclude that following uptake into the body, some styrene enters a fat depot in the body and release of styrene from here will occur for at least a few days post-exposure. Where metabolites of styrene have been studied in the urine of workers after a weekend free of exposure, some individuals have failed to clear styrene completely over this 48-hour period (Cherry and Gautrin, 1990). Conversely, in another study there was no increase in average urinary concentrations of mandelic or phenylglyoxylic acids with repeated daily exposures during the the working week (Pekari *et al*, 1993). However, other studies have indicated that following exposure, styrene is eliminated from the body over a period of several days (Engstorm *et al*, 1978a and 1978b). Overall, it appears that styrene persists in the body in humans for several days, suggesting that there would be some modest accumulation in fatty tissue on repeated exposure.

There have also been a number of studies that have assessed the presence of DNA adducts in humans exposed to styrene. These are described in detail in section 4.1.2.7.1

In a study investigating the influence of genetic polymorphisms on urinary metabolites of styrene, pre- and post shift urine samples were collected from 56 volunteers employed at

either a polyester resin factory or in glass-fibre reinforced plastic manufacture (De Palma *et al*, 2001). The genetic polymorphisms for styrene-metabolising enzymes (glutathione transferases GSTM1, GSTT1, GSTP1 and epoxide hydrolase EPHX) were characterised from peripheral blood. The quantity of one group of urinary metabolites, phenylhydroxyethyl mercapturic acids (PHEMAs), was higher in subjects with GSTM1pos genotype; other metabolites of styrene, mandelic acid (MA) and phenyl-glyoxylic acid (PGA) were less strongly affected. Workers with a GSTM1pos genotype produced around 5-6 times more urinary mercapturic acids that GSTM1null workers. These results indicate that some genetic polymorphisms for some styrene-metabolising enzymes alter the rate of removal/elimination of styrene oxide and other styrene metabolites.

Serial urine samples taken from two workers accidentally exposed to high levels of a solvent mixture containing styrene showed that differences in genotypes of styrene-metabolising enzymes (GSTM1, GSTT1, GSTP1, EPHX 113 and EPHX 139) would lead to different profiles and rates of elimination of mandelic acid, phenylglyoxylic acid and 4-vinylphenol-mercapturic acid conjugate (Manini *et al*, 2002). These results support earlier work conducted on a larger group of workers (De Palma *et al* 2001).

Another cross-sectional study showed different profiles of peripheral blood lymphocyte DNA damage (Comet assay) related to polymorphisms of styrene-metabolising enzymes (Buschini *et al*, 2003) – see also Section 4.1.2.7.3.

4.1.2.1.3 In vitro/ex vivo studies investigating metabolic capability in experimental animals and humans and comparisons between them

Liver

In an *in vitro* study which used liver tissue samples from Wistar rats, it was determined that styrene was oxidised to styrene oxide, which was subsequently hydrated to styrene glycol (phenyl glycol) (Belverdere *et al*, 1984). It was also shown that levels of GSH in the hepatocytes and S9 fractions used were depleted in the presence of styrene, due to the utilisation of GSH in conjugation reactions.

In the series of *in vitro* studies, the role of P450 isozymes in the metabolism of styrene to styrene oxide in rat liver microsomes has been investigated using monoclonal antibodies (Nakajima *et al*, 1994a). The results of the study indicate that in the rat liver microsomes the metabolism of styrene involved at least four P450 isozymes.

Samples of microsomes from 31 Asian and 14 Caucasian human livers have been analysed in vitro with respect to the metabolism of styrene (Kim *et al*, 1997). Histopathologically normal liver samples were obtained from autopsies. Twenty-one Asian and 13 Caucasian liver microsome fractions were randomly selected for immunoblot assay of the content of P450 isoenzymes. Metabolic rates measured in the Asian liver microsomes were statistically significantly higher (by about 60%) than those measured in the Caucasian liver microsomes at the low styrene test concentration (0.09 mM). At the higher styrene concentration (1.8 mM), rates remained higher in the Asian microsomes than in Caucasian microsomes, although the difference at this concentration was smaller and not statistically significant. Immunoblot analysis revealed that the mean expression levels of CYP1A2 and CYP2B6 were about 3-fold higher in the Caucasian microsomal fractions than in Asian fractions, whereas levels of CYP2E1 were about 2-fold higher in Asian microsomal fractions than in Caucasians. Further analysis indicated that at the low styrene concentration the most active P450s involved in the metabolism of styrene were CYP2E1 and CYP2C8, whereas at the high styrene concentration the most active P450s were CYP2B6 and CYP2C8.

In another series of studies it was determined that in human liver hepatoma G2 cell cultures CYP2B6 and CYP2E1 were the most active P450s in the metabolism of styrene, with CYP1A2 and CYP2C8 also showing a small degree of activity (Nakajima *et al*, 1994b).

The metabolism of styrene to styrene oxide has also been measured *in vitro* in human liver microsomal preparations by Carlson *et al* (2000). Six samples of normal human liver tissue were collected during surgical procedure or autopsy, and microsomal fractions prepared. Microsomes were incubated with 2 mM styrene at 37°C for 20 minutes and the presence of styrene oxide was determined by HPLC. Results showed that human hepatic microsomes can metabolise styrene to styrene oxide. The overall activity of the human liver hepatocyte microsomes was reported as being about 1/3 of that previously measured by the same workers in mouse liver microsomes (Carlson, 1997a, 1997b and 1998). The same workers have also demonstrated that styrene metabolism in mouse liver microsomes is comparable to that in mouse lung microsomes – see below (Carlson 1997a, 1997b and Carlson *et al*, 1998).

Another study has been conducted to compare rates of hepatic metabolism across species *in vitro*, using microsomes prepared from rat, mouse and human liver samples; human liver preparations were from samples selected for transplantation from 5 fatal accident victims. The V_{max} for conversion of styrene to styrene oxide was similar in the rat and mouse but 5 times lower in the human tissue (Mendrala *et al*, 1993). The Km for this conversion was similar across these three species. The Km of epoxide hydrolase for styrene oxide was lower in rat than in mouse and in human samples the Km was very low in comparison with rat or mouse, indicating a higher affinity for substrate. The V_{max} for epoxide hydrolase was the same across species. These results offer evidence of species differences; the tendency suggested by the results is for styrene oxide to be formed less readily and hydrolyzed more rapidly in the liver of humans compared with that of rodents.

In a series of studies by Nakajima *et al*, (1994b), the rates of formation of styrene glycol (phenyl glycol) from styrene in human, rat and mouse liver microsomes have been compared. At the low styrene concentration (0.085 mM) the mouse microsomes showed the greatest metabolic rate, followed by the rat and then the human. At the higher styrene concentration (1.85 mM) the metabolism was greater in the rat, followed by the mouse and then the human.

Respiratory tract

In a series of *in vitro* studies, the role of P450 isozymes in the metabolism of styrene to styrene oxide in rat lung microsomes has been investigated using monoclonal antibodies (Nakajima *et al*, 1994a). The results of this study indicated that in rat lung microsomes, only one P450 (CYP 2B1) displayed any significant activity. However, other studies (see below) have produced different findings.

The potential for metabolism of styrene to styrene oxide has been assessed *in vitro* in human lung microsomal preparations by Carlson *et al*, (2000). Six samples of normal human lung tissue were collected during surgical procedure or autopsy, and microsomal fractions prepared. Microsomes were incubated with 2 mM styrene at 37°C for 20 minutes and the presence of styrene oxide was determined by HPLC. Results showed that human lung microsomes appeared to lack any ability to metabolise styrene to styrene oxide (Carlson 1997a, 1997b and Carlson *et al*, 1998).

The apparent absence of cytochrome P450-mediated metabolism of styrene in 2 samples of human lung microsomes has also been reported by Filser *et al* (1999). However, some such activity, albeit at a very low level, was reported in a study involving lung microsomes

prepared from tissue samples taken from 38 patients undergoing lobectomy or pneumectomy (Nakajima *et al*, 1994b). This study focussed on the detection of styrene glycol (phenyl glycol) as a final product of cytochrome P450-mediated metabolism of styrene; the maximum rate of activity detected (~12 pmol/min/mg protein) was 380/360 times lower than that measured in comparable studies using lung microsomes from rats and mice (Filser *et al*, 1999).

Rates of metabolism in nasal tissue, and cytochrome P450 distribution in nasal and lung tissue, in rat, mouse and human nasal and lung tissues have been investigated *in vitro* (Green, 1999c; Green, 2000a; Green *et al*, 2001a and Green *et al,* 2001b). The nasal tissues of 40 male Sprague Dawley rats or of 100 CD-1 mice were pooled and S9 and microsomal fractions prepared. Nine morphologically normal human nasal tissue explants were obtained from the Royal National Throat, Nose and Ear hospital in London, UK, in compliance with local ethical guidelines, from patients with no recent histories of drug intake or radiotherapy. The tissue samples, which contained both respiratory and olfactory epithelium, were removed during surgery and immediately frozen in liquid nitrogen and stored at –70°C until use. One sample was used to prepare a microsomal fraction for analysis and the remaining 8 samples were pooled and used to prepare an S9 fraction. All S9 fractions, from each species, were dialyzed to remove glutathione prior to analysis. The metabolic rates of styrene in each tissue fraction were determined. Tissue fractions were also analysed using chemical inhibitors of specific cytochrome P450s in an attempt to identify the major isoforms involved in styrene metabolism. The distribution of cytochrome P450s 2E1 and 2F2 (the two isoforms that were shown in these studies to be predominant in styrene metabolism) was also determined in the nasal and lung tissues of the above species using immunohistopathological techniques.

In the microsomes prepared from rat and mouse olfactory and respiratory nasal epithelium tissue samples, the rates of metabolism of styrene to styrene oxide were similar between the two species (7.46 and 6.59 nmol SO/min/mg protein in the olfactory epithelium of rats and mice respectively); in each species the metabolic rates in the respiratory fractions (3.25 and 3.64 nmol SO/min/mg in rats and mice respectively) were approximately half those observed in the olfactory fractions. In human nasal tissue fractions (microsomal and S9) no styrene metabolism was detectable (limit of detection 0.04 nmol SO/min/mg protein), although some CYP2E1 and 2F2 was identified. In the lung samples, both CYP2E1 and CYP2F2 were expressed in significantly larger amounts in the mouse than in the rat; neither enzyme was detected in the human lung samples. However, it should be noted that it is unclear whether or not the antibody used for the immunostaining of the murine CYP2F2 had been able to detect those CYP2F enzymes, which are the orthologous forms of CYP2F2 in humans (CYP2F1). There were differences between rat and mouse in the distribution of the two enzymes along the length of the lower respiratory tract, but in terms of the cell type the enzymes were found exclusively in the non-ciliated Clara cells.

In other investigations, CYP2E1 and CYP2F1 (the human correlate of the murine CYP2F2) could be identified in human respiratory tract and lung tissue by using different techniques; mRNAs encoding for CYP2E1 and CYP2F1 were detected in primary cultures of human bronchial cells from several donors (Runge et al., 2001).

Bernauer et al (2005) found that CYP2E1 activity was present among 86 samples of human lung tissue at levels (range between 1.1 and 23.9 pmol/mg protein/min) which are 3 orders of magnitude lower than those reported for the human liver (in the range of nmol/mg protein/min). Overviews of the expression of xenobiotic-metabolising CYP enzymes in human lung tissues have been published by Ding and Kaminsky (2003) and by Hukkanen et al (2002).

In another study, using microsomes prepared from mouse, rat and human (n=38) lung tissues, quantitative Km and V_{max} values for the conversion of styrene to styrene oxide by cytochrome P450-dependent monooxygenases have been determined (Filser *et al*, 1999). In CD-1 mice the Km was 0.0013 mmol/l, the V_{max} was 4.5 nmol/min/mg protein and the V_{max}/Km was 3440 µl/min/mg protein, indicating that in the mouse lung cytochromes have a relatively high activity with respect to the metabolism of styrene to styrene oxide. In Sprague-Dawley rats the Km was 0.012 mmol/l, the Vmax was 4.7 nmol/min/mg protein and the V_{max}/Km was 398 µl/min/mg protein, indicating that in the rat lung the P450 cytochromes have a moderate (relative to mouse lung) ability to metabolise styrene to styrene oxide. No activity was detectable in human lung microsomes.

In the same study Km and V_{max} values for the detoxification of styrene oxide via both the epoxide hydrolase (EH) and glutathione-S-transferase (GSH) pathways were also determined. In CD-1 mice the GSH pathway was more prominent in the metabolism of styrene oxide but the ability to detoxify styrene oxide via either the EH or GSH pathways was poor.

In the rat lung microsomes, both pathways had approximate equal prominance in the metabolism of styrene oxide and both pathways had a relatively moderate ability to detoxify styrene oxide.

In human lung microsomes no metabolism of styrene to styrene oxide was detectable. The EH pathway was more prominent than the GSH pathway in the metabolism of added styrene oxide and both pathways had a relatively moderate ability to detoxify styrene oxide. Given the apparently very low potential of human lung tissue to generate styrene oxide these results indicate that in humans the potential for the build-up of styrene oxide in the lung is very low.

In another *in vitro* study, the activity towards styrene oxide of epoxide hydrolases and glutathione S-transferases in the olfactory and respiratory fractions of the rat and mouse nasal tissue and in the combined fraction of human morphologically normal nasal tissue (n=6) was compared (Green, 2000c). For both enzymes and both regions of the nasal epithelium, metabolic activity was appreciably (2-10 fold) greater in rats than in mice. The activity of epoxide hydrolases in the human tissue was quite variable, but of the same order as in rodents; glutathione S-transferase activity was not reliably measured.

In a study to investigate the metabolism of styrene to R- and S-styrene oxide (SO) in rats and mice, microsomal fractions from whole liver and whole lung homogenates were prepared (Hynes *et al*, 1999). The effect of various cytochrome inhibitors on R- and S-SO formation in Clara cells and Type II lung cells was also determined.

The liver and lung samples from mice produced more of the R-enantiomer of SO than S-SO, and for rats more S-SO was formed than R-SO. α-Naphthaflavone, and α-methylbenzylamino-triazole, specific inhibitors of cytochrome P450, CYP1A and CYP2B respectively, had little or no effect on the metabolism of styrene in mouse Clara cells. These results imply that, in mice, CYP1A and CYP2B have minimal roles in styrene metabolism. The addition of 5-phenyl-1-pentyne, an inhibitor of CYP2F2, caused a statistically significant reduction in the production of R- and S-SO in mouse lung cells, indicating that this enzyme does have a key role in the metabolism of styrene in mice. Furthermore, the extent of styrene metabolism was greater amongst the Clara cell-rich samples than those rich in Type II cells, indicating that the former are more important in the metabolism of styrene. Diethyldithio-carbamate (DDTC), a specific inhibitor of CYP2E1, had no effect on styrene oxide production in mouse Clara cells indicating that this isozyme has also no role in styrene metabolism in mouse lung.

In a study conducted to investigate strain and sex differences in the detoxification of styrene oxide via epoxide hydrolase to yield styrene glycol, hepatic and pulmonary microsomal

preparations from non-Swiss albino (NSA) and Swiss CD-1 mice were compared for their abilities to metabolise racemic, S- and R-styrene oxide (Carlson, 1999b). Epoxide hydrolase activity was higher in the liver than in the lungs. In incubations with racemic styrene oxide, R-styrene glycol formation was predominant. Only minor strain differences in the epoxide hydrolase activity were found. However, while the oxidation of styrene to styrene oxide was similar in male and female NSA mice, male hepatic microsomes were more active in the metabolism of the oxide to the glycol. Hepatic metabolism of styrene oxide to styrene glycol was inducible by butylated hydroxyanisole, whereas pulmonary metabolism was not. These data indicate that strain differences in the detoxification of styrene oxide via epoxide hydrolase in mice are minor, but that there are sex differences.

Carlson (2000) investigated the detoxification of styrene oxide via epoxide hydrolase to yield styrene glycol in enriched Clara-cell preparations isolated from male CD-1 mice. In incubations with racemic styrene oxide, R-styrene glycol was the predominant metabolite with an R/S ratio of 3.6. In incubations with the pure styrene oxide enantiomers, the corresponding styrene glycols were produced. Epoxide hydrolase activity was slightly higher with the S-styrene oxide than with the R-styrene oxide. Addition of reduced glutathione to the incubation medium resulted in an increase in epoxide hydrolase activity, perhaps by decreasing oxidative stress.

In a comparative study of rats and mice conducted to determine styrene oxide (SO) lung burdens, freshly prepared lungs of male Sprague-Dawley rats and male $B_6C_3F_1$ mice were ventilated with styrene vapours of defined concentrations (40-980 ppm for rats and 40-425 ppm for mice) (Filser, unpublished, 2004). The lungs were then perfused *in situ* for 10 min and SO measured in the effluent perfusate by CG/MS.

In both species, SO levels increased with increasing levels of styrene. From the graphically presented data, it can be estimated that equivalent lung burdens of SO (~0.45 nmol/g tissue) occurred at around 160 and 1000 ppm styrene in mice and rats respectively. However, data from long-term inhalation studies have shown that lung tumours develop at styrene concentrations $\geq$ 20 ppm in mice (Cruzan *et al*., 2001), and no tumours are found in lungs of rats exposed to styrene concentrations of up to 1000 ppm (Cruzan *et al*., 1998). Thus, it is possible to deduce that SO is not the main metabolite responsible for tumour formation. It should be noted that the SO lung burdens measured in this study were aggregate measures of lung SO concentrations and do not necessarily represent the levels of SO in individual Clara cells.

In a recent *in vitro* study (Bartels et al, unpublished, 2004) conducted to further characterise styrene metabolism (especially the pathway involving initial hydroxylation to 4-VP) and to investigate 4-VP metabolism identifying its major downstream metabolites and determining species differences, styrene and 4-vinylphenol (4-VP) were incubated, separately, with microsomal fractions of liver and lung tissue from mice, rats and humans. Microsomes of liver and lung tissue obtained from male CD1 mice, male SD rats and human donors (50 and 4 donors of mixed genders for liver and lung samples respectively) were incubated with 0.01, 0.1 or 1.0 mM styrene or with 0.05 or 0.5 mM 4-VP. Selected mouse styrene or 4-VP incubations were used to determine the metabolic production of CO_2. In addition, selected styrene incubations were analysed for the production of 4-VP and selected 4-VP lung microsomal incubations were performed in the presence of excess glutathione (GSH) in order to trap reactive 4-VP metabolites.

No significant production of CO_2 was detected following incubation of styrene or 4-VP with microsomes of mouse liver or lung tissue indicating that the liver or lung are not the primary site of aromatic ring cleavage of styrene in the mouse. Substantial species differences were observed in the rate of styrene metabolism in both liver and lung microsomes, with the mouse having the highest styrene conversion (76-80%) compared to the rat (18-29%) and the human donors (not detected[ND]-26%). Following incubation with styrene, only trace

levels of 4-VP were found in lung microsomes of both mice (ND-0.015% of substrate) and rats (ND-0.019%). No measurable 4-VP was detected in human lung microsome incubations. No detectable 4-VP was found in liver microsomes of either mice, rats or humans. These results suggest that conversion of styrene to 4-VP is a minor route of styrene metabolism *in vitro* and that the small amount of 4-VP produced is subsequently rapidly metabolised. Incubations of 4-VP with mouse lung microsomes produced two major and several minor reactive metabolites. By incubating these mouse lung microsomes with 4-VP in the presence of excess GSH, the two major metabolites of 4-VP were isolated. These were identified as the GSH conjugates of the side-chain epoxide and the ring-hydroxylated (4-VP hydroquinone) derivatives of 4-VP. The rate of formation of these two 4-VP downstream products was then determined from incubations of styrene with lung microsomes from mouse, rat and human donors in the presence of excess GSH. Relative formation rates for both 4-VP derivatives were highest in mouse lung microsomes (123.35 and 0.325 pmoles/min/mg of 4-VP epoxide GSH conjugate and 4-VP hydroquinone GSH conjugate respectively). Yields of 4-VP epoxide and 4-VP hydroquinone respectively were 79% (97.2 pmoles/min/mg) and 14% (0.045 pmoles/min/mg) lower in rat lung microsomes compared to mouse lung microsomes. Incubates from human lung microsomes contained only 5% (6.4 pmoles/min/mg) of 4-VP epoxide and 1.5% (0.005 pmoles/min/mg) of 4-VP hydroquinone concentrations measured in mouse lung. These results indicate that in human lung the reactive downstream metabolites of 4-VP are produced to a lesser extent than in rat and mouse lung. Some caution needs to be exercised, as these are *in vitro* studies; however, at present, it is not technically possible to perform this kind of study *in vivo* due to the extreme reactivity of these downstream metabolites.

4.1.2.1.4 Development of a physiologically-based pharmacokinetic (PBPK) model and prediction from its use

A PBPK model has been developed and used to predict blood and expired air styrene concentrations in humans inhaling styrene, and the generation and removal of styrene oxide by the liver, and in the lung, in mice, rats and humans exposed to styrene (Csanady *et al*, 1994; Filser *et al*, 1999). The model can predict blood styrene and styrene oxide levels that accurately reflect the available experimental results, both within and between species, including humans (Csanady *et al*, 1994). In more recent work, the model has been used to predict the generation and removal of styrene oxide in the lungs, the consequences for lung glutathione (GSH) levels and the implications if it is presumed that depression of GSH levels can lead to cytotoxicity. The model predicts the following scenarios in mouse, rat and human, and comparisons between them:

As a result of exposure to styrene, there would be a significantly higher styrene oxide burden in lungs of mice compared to rats and, especially, to humans. The glutathione S-transferase reaction of styrene oxide with GSH depletes this co-enzyme in the lungs of mice exposed to styrene concentrations of 80 ppm and above. Prolonged repeated low levels of GSH, as has been demonstrated for an exposure concentration of 160 ppm styrene, may result in an imbalance of the “thiol redox system” and consequently in continuous cytotoxicity leading to cell proliferation. In the rat lung, less pronounced effects are to be expected, since a significant loss of GSH requires styrene exposures of at least 300 ppm. In the human lung GSH loss is most improbable for two reasons. First, styrene oxide concentrations resulting from styrene exposure are predicted to be by far smaller than in equally exposed rodents. Second, styrene oxide metabolism in the human lung is catalysed mainly by epoxide hydrolase and not by glutathione S-transferase, the activity of the latter being very low. Consequently, in the lung of styrene-exposed humans, cytotoxicity due to GSH depletion should not occur.

A further PBPK model has been developed to extend previous work. This models the absorption, distribution and metabolism of styrene and incorporates production and clearance of styrene oxide from the respiratory tract, with detailed modelling of the terminal bronchioles in rats, mice and humans (Sarangapani *et al,* 2002). The respiratory tract was modelled using a 3-layer structure – the lumen, epithelial cell layer, and submucosal layer; this is obviously a simplification of the range of different cell types involved but is typical of this sort of modelling. However, the model incorporated a Clara cell-rich bronchiolar compartment to reflect the importance of these cells in styrene metabolism. The epithelial component was taken to be the site of styrene and styrene oxide biotransformation. The other main sites of metabolism were the liver, nasal cavity and bronchioles. Most physiological parameters were taken from literature (such as partition coefficients), although a small number of non-critical parameters were estimated (for example the thickness of the conducting airways submucosal epithelium). A range of data from chamber studies to measured concentrations of styrene and styrene oxide in tissues were used to validate the dose metrics for mice, rats and humans. In most cases the model fit to the experimental data was very good, although occasionally modifications were needed (for example alveolar ventilation rates were reduced by 30% of the usual mean value in order to fit mouse chamber study data; apparently the change in ventilation rate is typical of closed-chamber studies in rodents).

The authors showed that R-SO predominates in the terminal bronchioles of mice, while S-SO predominates in rats. The model predicts a maximum level of 2 μM R-SO in the terminal bronchioles of rats due to saturation of styrene metabolism at 500-600 ppm. This is only half the level of R-SO achieved in the terminal bronchioles of mice exposed to 20 ppm styrene. The Sarangapani *et al*. model predicts that at a given airborne concentration, the level of total SO in the terminal bronchioles of mice is approximately 10-fold higher than in the terminal bronchioles of rats and 100-fold fold than in humans. In humans, the maximum concentration of SO in the terminal bronchioles (approx. 0.09 μM) is reached at an airborne concentration of 200 ppm; this same concentration is found in the lungs of mice exposed only to 0.1 ppm styrene.

In an extension of their earlier PBPK model, Csanady et al. (2003) developed a new model to assess the lung burden in styrene-exposed mice, rats and humans. The model incorporated the pulmonary metabolism of styrene and styrene oxide (SO), the prediction of glutathione levels following styrene exposure together with the formation of haemoglobin- and DNA-adducts. The lung was represented by two compartments: the conduction zone and the alveolar zone. The conduction zone modelled the nose together with the upper respiratory tract including the bronchi and bronchioles, while the alveolar zone modelled the alveoli and the corresponding capillary endothelium. All the physiological and biochemical parameters were similar to those of the earlier model, except for the value of the cardiac output fraction perfusing the adipose tissue of rodents, which was changed from 9% to 1.7% to reflect reported measured data (Csanady et al., 1994). As a result of the change, the metabolic clearance was lowered to balance the metabolic rates.

The model predicted glutathione depletion in mice exposed to styrene concentrations as low as 30 ppm; in contrast, rats were shown to have less glutathione depletion, while no glutathione depletion was predicted in human lungs at styrene concentration of up to 200 ppm. The simulation values predicted for SO lung burden in rat and mouse following styrene exposure were similar to values observed in experimental findings. The highest SO concentration was predicted for mouse lung, followed by the rat lung with the human lung having the lowest SO burden. Exposure to 20 ppm styrene produced a 3-fold higher SO concentration in mouse lung than in rat lung. However, at higher exposure concentrations, the difference in SO lung burden between mice and rats was predicted to flatten. At 70 ppm, this difference was reduced to less than 2-fold, and at 160 ppm the SO lung burden in mice was only 1.6 times higher than that observed in rats. The pulmonary SO burden in humans

exposed to 20 ppm of styrene for 8-hours was predicted to be 17- and 50- fold lesser than the corresponding values in rats and mice.

The kinetics of systemically available styrene and SO following oral exposure were also modelled. Up to styrene concentrations of 250 ppm, the toxicokinetic processes followed first order kinetics, hence, SO blood levels were similar in both the mouse and rat. At higher styrene concentrations, SO production reached a plateau in the rat, whereas in the mouse, SO blood levels increased with increasing styrene concentration such that at an exposure of 800 ppm, SO blood levels in the mouse were 20 times higher than the predicted values in the rat. The kinetics of styrene in humans were predicted to be similar to those of the rat. The model also confirmed experimental findings that SO is more potent than styrene in forming adducts with haemoglobin (Hb) and DNA. Assuming that the rate constant describing haemoglobin adduct formation in rats is representative of the human rate constant, the model predicted a steady state adduct level of 37pmol/g Hb for an occupational exposure (8h/d, 5d/wk) to 20 ppm styrene.

4.1.2.1.5 Summary of toxicokinetics

A substantial amount of information is available on the toxicokinetics of styrene in humans, following exposure by the inhalation route; information on percutaneous absorption in humans is also available.

In humans, inhaled styrene vapour (at concentrations of 10-200 ppm) is well absorbed across the respiratory tract. Thus, a value of 100% for absorption via the inhalation route of exposure is taken forward to the risk characterisation.

Dermal absorption of the liquid has been estimated to be approximately 2% of the applied dose in an *in vitro* study using human skin samples. This value is taken forward to the risk characterisation. Dermal uptake of the vapour appears to make only a small contribution (5% or less) to the total body burden arising from combined inhalation and dermal exposure to the vapour.

No information is available on oral absorption in humans, but from the physicochemical properties of styrene and experimental animal information, one would expect extensive absorption from the gastrointestinal tract. Thus, a value of 100% for oral absorption is taken forward to the risk characterisation. Following absorption, it can be predicted from experimental animal data that styrene is widely distributed in humans, and needle biopsy investigations have shown that styrene certainly locates in adipose tissue; there was a correlation between the amount of body fat and the total body burden of styrene. Data on styrene blood levels in human volunteers following single inhalation exposures and in rats exposed via inhalation show that at identical exposure concentrations, styrene blood levels are very similar (e.g. 2.5 and 3.5 μg/ml in rats and humans respectively at 100 ppm styrene).

The rate of absorption following inhalation is much higher (2-3 fold) in mice than in rats. The absorption rate in humans is approximately the same as in rats. The rate of styrene uptake in the upper respiratory tract is partly dependent on its metabolism, and was decreased when animals were pre-treated with a P450 inhibitor.

In humans, styrene is eliminated from the body relatively rapidly, primarily in the urine. However, there is some evidence for modest biopersistence in human adipose tissue on repeated daily exposure. Styrene clearance from blood is biphasic. Half-lives for inhaled styrene were reported at 0.6 hours for the first elimination phase and 13 hours for the second elimination phase. From studies in mice, there is evidence that styrene is also rapidly eliminated from blood following either single or repeated inhalation exposure. A study in pregnant mice has shown that styrene and/or its metabolites can cross the placenta into the foetus.

The metabolism of styrene has been studied thoroughly in mice, rats and humans. A number of metabolic pathways have been identified (Figure 1). The evidence suggests that these pathways are active in mice, rats and humans, although there are species differences in their relative importance.

Styrene is metabolised extensively in all species. According to a PBPK model developed by Ramsey and Andersen, saturation of styrene metabolism in humans occurs at blood levels exceeding 1.7 μg/ml styrene or 200 ppm styrene in air. Below these concentrations, the rate of styrene metabolism is limited by the rate of blood perfusion in liver or other organs involved in styrene elimination. The first step in the metabolism of styrene involves oxidation of the aromatic ring or side-chain. The main route in each species is the oxidation of the side chain to give the epoxide, styrene-7,8-oxide (SO). A number of studies have demonstrated the involvement of P450 in this step and have provided information on the specific P450 isoforms involved in the production of SO (CYP2E1, CYP2B6 and CYP2C8 in the liver and CYP2F2/1 and CYP2E1 in the lung). Pre-treatment of rodents with diethyldithiocarbamate, a specific cytochrome P450 monooxygenase inhibitor, effectively inhibited the metabolism of styrene, and reduced toxicity in the mouse lung and nasal tissue was observed when the animals were pre-treated with 5-phenyl-1-pentyne, a cytochrome P4502F2 inhibitor. In isolated Clara cells and microsomes, an inhibitor of 2F2 reduced the production of SO by around 30-50%; a 2E1 inhibitor showed less inhibition indicating a lower importance of this isoform in the lung. The SO produced is enantiomeric and is produced in the R- and S-forms, probably as a result of metabolism by different P450 isoforms. Different ratios of R-SO to S-SO are found in different tissues and different species. Mouse Clara cells produce about 3 times more of the R-enantiomer than the S-enantiomer, while rat produces more of the S-enantiomer, and humans, like rats, produce more of the S-form.

SO is either metabolised further by conjugation with glutathione to give mercapturic acids, or is hydrolysed by epoxide hydrolase (EH) to phenylglycol. This is subsequently metabolised to mandelic, phenylglyoxylic and hippuric acids. P450 and EH are both microsomal enzymes in the endoplasmic reticulum. Therefore, SO produced *in situ* by P450 may potentially be rapidly detoxified if there is sufficient EH present.

Other metabolic pathways can lead to phenylacetaldehyde (PA) and phenylacetic acid (PAA) (via side-chain β-oxidation and hydroxylation), to phenylethanol and acetophenone (via side-chain α-oxidation and hydroxylation), oxidation of the aromatic ring to give 4-vinylphenol (4-VP), and products of ring opening. These metabolites are excreted in the urine. There are studies which have demonstrated that P450 enzymes are also involved in both the side-chain and ring oxidation of styrene and that 4-VP is further metabolised in lung microsomes by specific P450 isoforms to extremely reactive downstream products (e.g. an epoxide and a hydroquinone derivative). Subsequently these derivatives are conjugated with glutathione, but at present there is no information on the relative rates of 4-VP metabolites detoxification between different species.

There are some data from rodents (rats and mice) and humans to indicate the relative extent of flux through these various pathways. The approximate relative contribution of each metabolic pathway in each species, as determined from urinary metabolites, is shown in Table (a). The urinary metabolites are an indication of the overall metabolism of styrene, which is considered to occur largely in the liver.

It is clear that metabolism involving SO as an intermediate is a major route in rodents and humans. However, there are some notable species differences. In humans, almost all of styrene (95%) is metabolised to SO and further metabolised by EH; approximately 5% of styrene is metabolised via the phenylacetaldehyde pathway. No more than trace amounts of SO-GSH conjugates or ring-oxidized metabolites of styrene (4-VP) occur in humans exposed to styrene. Further metabolism of SO by EH is important but less extensive in rodents than in humans (68-72% in rats and 49-59% in mice). In rodents, conjugation of SO with GSH is an important route accounting for up to a third of the SO removal. The most

significant difference between mice and rats is in relation to the production of phenylacetaldehyde (12-22% in mice against 3-5% in rats) and products of ring-oxidation (4-VP; 4-8% in mice against <1% in rats).

Table (a). Approximate relative contribution of metabolic pathways for styrene indicated by urinary metabolites (Cruzan *et al.*, 2002; Johanson *et al.*, 2000)

Metabolic route	**Urinary metabolites (%)**		
	Rat	**Mouse**	**Human**
Products of action of EH on SO	68-72	49-59	95
Conjugation of SO with GSH	23-26	20-35	Very low
Phenylacetaldehyde	3-5	12-22	5
Ring opening	ND	ND	<1
Products of 4-vinylphenol conjugation	<1	4-8	<1

ND, not determined.

Metabolism in specific tissues

The metabolism of styrene in specific tissues, lung, liver and nasal tissue, has been investigated in detail. These studies have demonstrated significant differences in the metabolism of styrene between these tissues and between species.

Liver

Styrene is metabolised to a significant extent in the liver in all species. Results from PBPK modelling suggest that metabolism of styrene by the liver is probably mainly responsible for the production of the blood level/body burden of SO. These models predict that following inhalation exposure to styrene concentrations of up to 250 ppm, the toxicokinetic processes follow first order kinetics, hence, SO blood levels are similar in both the mouse and rat. At higher styrene concentrations, SO production reaches a plateau in the rat, whereas in the mouse, SO blood levels increase with increasing styrene concentration such that at an exposure of 800 ppm, SO blood levels in the mouse are 20 times higher than the predicted values in the rat. The kinetics of styrene in humans are predicted to be similar to those of the rat. The enzyme kinetics for hepatic P450 and EH suggest that human liver has a 3-5 times lower ability to produce SO and a higher ability to hydrolyse it with EH than rodents, resulting in half-lives for SO hydrolysis of 38, 9-17 and 1.8 min for mice, rats and humans, respectively. Human liver microsomes produced approximately equal proportions of R- and S-SO.

Lung

The available data suggest that the Clara cell is mainly responsible for metabolising styrene in the lung. In cells isolated from the lungs of mice and rats it was evident that the Clara cells were responsible for the metabolism of styrene, and that type II cells did not metabolise it to

any significant extent. This is consistent with the fact that Clara cells contain the main metabolising enzymes, 2E1 and 2F2/1, and type II cells do not (Forkert, 1995; Buckpitt et al., 1995).

There are species differences in the number and structure of Clara cells. In mice, Clara cells are relatively numerous (approx. 89% of bronchiolar epithelium) and are spread throughout the airways which will impact significantly on the metabolism of styrene. In rats, they are significantly fewer in number (approx. 25% of bronchiolar epithelium) and they are found mainly in the terminal bronchiolar region. In contrast to rodents, in the human lung, Clara cells are rare and are found in small numbers in the distal bronchioles. They also are morphologically different, not possessing the extensive endoplasmic reticulum (on which P450s are localised) that is apparent in mouse Clara cells (Plopper et al., 1980). However, it should be noted that in humans the pulmonary cell types with the most significant CYP expression are cells of the bronchial and bronchiolar epithelium and not the Clara cells (Foth, 1995), and that cell-type specific expression of CYP enzymes in human lung has not been thoroughly investigated up to now. The distribution of Clara cells was mirrored by the pattern of immunohistochemical staining for two isoforms of P450, 2E1 and 2F2. In the mouse, these were found at high levels in the terminal bronchioles and to a lesser extent in the larger bronchioles. In rats, the activity of these enzymes was found at only low levels in the terminal bronchioles, and in humans, it was below the level of detection in one study (Green,2000a) and it was found at levels (~12 pmol/mg protein/min) 400 times lower than those in mice in another study (Nakajima et al., 1994b). A more recent investigation (Bernauer et al., 2005) has reported that CYP2E1 activity was found among 86 samples of human lung tissue at levels (range between 1.1 and 23.9 pmol/mg protein/min) which are 3 orders of magnitude lower than those reported for the human liver (in the range of nmol/mg protein/min). There are also other studies, in which the expression of CYP2F1 in human lung tissue has been demonstrated (Runge et al., 2001; Hukkanen et al., 2002; Ding and Kaminski, 2003).

Studies in microsomes from lung cells have indicated significant species differences in the rate and extent of both the production of SO, its subsequent detoxification and in the production of the ring-oxidized metabolite, 4-VP and its downstream products. The enzyme kinetics for P450 in microsomes indicated that mouse and rat microsomes metabolise styrene to SO at approximately the same rate at high concentrations (i.e. at saturation) but, due to the much lower K_m for mouse P450, mice will produce SO around 3-10 times more rapidly than rats at lower concentrations. The maximum rate of hydrolysis by EH is similar in rats and mice, but it occurs to a greater extent in rats at lower concentrations due to the lower K_m. The extent of SO conjugation with GSH was similar in mice and rats. Recent investigations have also shown that styrene is metabolised to 4-VP which is further metabolised to reactive downstream products, an epoxide and a hydroquinone derivative, and that in rats these reactive 4-VP derivatives are produced to a lesser extent than in mice (14-79% of the mouse concentrations). In marked contrast, in most human microsome samples tested there was no styrene-metabolising activity, although in some individual samples, very low levels (around two orders of magnitude lower than in mouse and rat microsomes) of styrene metabolism to SO and styrene glycol were detected. No detectable levels of styrene metabolism to 4-VP were also found, most likely as the consequence of the high reactivity of 4-VP. Indeed, in experiments conducted in the presence of excess GSH to trap the reactive downstream metabolites of 4-VP, the epoxide and the hydroquinone derivatives of 4-VP were isolated. However, these represented only 1.5-5% of the concentrations determined in mouse lung. Human microsomes also had a substantially higher EH activity than rodents, indicating that any SO produced *in situ* or migrated from the blood would be rapidly hydrolysed.

In addition to differences in the extent and rate of production of SO, there are also clear differences in the ratio of R- and S-SO formed. Mouse Clara cells produce approximately 2-4 times more R-SO than S-SO, whilst rats produce a more equal ratio or preferentially S-SO.

Using PBPK modelling, it has been shown that R-SO predominates in the terminal bronchioles of mice, while S-SO predominates in rats. The Sarangapani model predicts a maximum level of 2 μM R-SO in the terminal bronchioles of rats due to saturation of styrene metabolism at 500-600 ppm. This is only half the level of R-SO achieved in the terminal bronchioles of mice exposed to 20 ppm styrene. The model also predicts that at a given airborne concentration, the level of total SO in the terminal bronchioles of mice is approximately 100-fold higher than in the terminal bronchioles of humans. In humans, the maximum concentration of SO in the terminal bronchioles (approx. 0.09 μM) is reached at an airborne concentration of 200 ppm; this same concentration is found in the lungs of mice exposed only to 0.1 ppm styrene. Similarly, the Csanady model predicts that the pulmonary SO burden in humans exposed to 20 ppm of styrene for 8-hours is 17- and 50- fold lesser than the corresponding values in rats and mice. This model also predicts that glutathione depletion occurs in the lungs of mice exposed to styrene concentrations as low as 30 ppm; in contrast, rats are predicted to have less glutathione depletion while no glutathione depletion is estimated to occur in human lungs at styrene concentrations of up to 200 ppm.

Nasal tissues

In rats and mice, the uptake of styrene in the upper respiratory tract is partly dependent on its metabolism. The percentage of styrene absorbed decreased with increasing airborne concentration, demonstrating saturation of metabolism. Saturation of uptake occurred at a lower airborne concentration of styrene in rats than in mice, indicating a greater metabolic capacity in mice than in rats. From investigations conducted on microsomes from olfactory and respiratory nasal tissue, it has been found that nasal olfactory epithelium produces SO from styrene at about the same rate in both rats and mice with an R:S ratio of about 3. There is approximately twice as much metabolism occurring in the olfactory cells compared to the respiratory region. However, there is a signficant difference in the activity of EH, with rats having up to 10-fold higher activity than mice. The K_m for EH was much lower in both species in respiratory epithelium than in olfactory epithelium, especially for R-SO. In contrast, human microsomes did not appear to have any ability to produce SO, even though 2E1 and 2F2 were found at low levels in some specific regions of the human nasal region. Human microsomes had around 3-times higher EH activity than mice.

There were some differences in the distribution of 2E1 and 2F2 within the nasal region in mice and rats. Mice generally had higher levels in the olfactory apical cytoplasm and basal cells.

Overall

These data indicate significant differences in the metabolism of styrene between species and between tissues. It should be noted that, although these data arise from *in vitro* studies and PBPK modelling, they clearly mirror the toxicodynamic picture of styrene obtained *in vivo*. The tissue-specific metabolism of styrene suggests that *in situ* metabolism within each tissue may be a more important determinant of toxicity than the overall systemic metabolism and blood levels of styrene metabolites. The implication of this is that the specifics of the local metabolism in a target tissue must be considered when extrapolating findings in animals to assess the likely hazard and risks in the equivalent human tissues.

A general observation is that the human tissues investigated – apart from the liver - produce very little SO, if any, and have a greater capacity to hydrolyse SO with EH than rodents. This difference is most pronounced in human nasal and lung tissues where production of SO is minimal or undetectable, and is also associated with a greater capacity to hydrolyse SO by EH. The mouse lung and nasal tissues produce the greatest amount of SO among the species tested, and, in general, have less EH activity, suggesting that significantly high local concentrations of SO will be present in these tissues. It is also evident that other toxic

metabolites, particularly 4-VP and its reactive downstream products, are produced to a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse concentrations). Although it cannot be ascertained whether or not these species differences in the formation of 4-VP metabolites in the lung may be a reflection of the different numbers of Clara cells (the metabolically active lung cells) present in the different species, since 4-VP metabolites are produced by the same cytochrome P450 enzymes involved in the production of SO, it is most likely that the species differences in the formation of 4-VP metabolites observed reflect species differences in metabolic capability.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Inhalation

Styrene is of moderate toxicity to rats and guinea pigs via single inhalation exposure. An inhalation LC_{50} value for styrene vapour of 2770 ppm (11.8 mg/1) in the rat (4-hour exposure) has been reported (Shugaev and Yaroslavi, 1969). The situation in mice is somewhat different (see below).

In a study by Spencer *et al* (1942), groups of rats and guinea-pigs were exposed to styrene at concentrations from 1449 to 10 672 ppm for up to 40 hours. Signs of clinical toxicity observed immediately from the onset of exposure in all animals were marked signs of irritation of the eyes and nasal mucosa (lachrymation, salivation, nasal discharge, violent scratching and rubbing). The severity of response increased with increasing exposure concentration. No other signs of toxicity were observed in any animals exposed to 1449 ppm for up to 30 hours. In animals exposed to 2139 ppm and above, general signs of CNS depression (weakness and unsteadiness) were observed in both rats and guinea-pigs. The onset of effects was more rapid and the severity of response increased (including deaths) with increasing exposure concentration. Necropsy of animals from exposure groups in which /some/ deaths occurred (2139 ppm and above), revealed changes in the lungs of both species. The changes varied from “slight” congestion to “total” congestion, hamorrhage, oedema, exudation and leukocyte infiltration. Effects were more severe in guinea pigs than in rats at equivalent exposure concentrations and durations of exposure.

In a study to investigate effects on the vestibulo- and opto-oculo motor system in the rat, nystagmus (eye movement) parameters were examined with exposure to styrene (Niklasson *et al*, 1993). Thirty-three pigmented rats (18 females and 15 males) were used in an initial control test without styrene exposure and then for 1-2 subsequent tests with 1 or 2 concentrations of styrene. Exposures were separated by intervals of at least 1 week. The concentrations used were 857-5190 ppm for exposure durations of up to 60 minutes. Testing began 10 minutes after initiation of exposure. The results from exposure were presented graphically only and no control data were presented. Nystagmus was elicited by vestibular stimulation in conflict with a visual input; there was an exposure-related decrease in the ability to cancel this effect. Saccades (quick eye movements) were elicited by touching the tail of the rat. During exposure to styrene, the eyes immediately started a slow drift after a saccade, in contrast to controls. These results are consistent with CNS depression at relatively high concentrations of styrene.

A study by Fechter (1993) explored the potential for a single exposure to styrene to affect auditory function in guinea pigs as the model system. These were exposed to either noise or styrene alone or the two insults combined. Exposure to styrene was to a single seven hour exposure to either 500 or 1200 ppm. Noise exposure was to 95 dB(A) white noise, which

results in a mild temporary auditory threshold shift for approximately one day post-exposure. The A weighting scale (dB9A)) maximises sound energy in the human auditory range.

Auditory function was assessed using electrophysiological techniques. Measurements were made at the round window of compound action potential and cochlear microphonic following stimulation by tone pips at selected frequencies. Compound action potential assesses inner hair cell, Type 1 spiral ganglion and outer hair cell function. The cochlear microphonic is primarily generated by the outer hair cell. Thus various components of the auditory sensorineuronal system were assessed.

Groups (n=5) of guinea pigs received either 500 ppm or styrene alone for seven hours, 95 dB(A) alone also for seven hours, or both the styrene and noise concomitantly for seven hours. Control animals underwent the same procedures but remained untreated. Auditory function was assessed at one day post-treatment. In those animals which received noise alone or noise and styrene together, an increase was observed in the auditory threshold for the induction of the compound action potential at 8, 12 and 16 kHz. This increase was of the same magnitude in both of these groups. Styrene exposure alone had no effect on auditory function as measured. Only a slight, non statistically significant increase in choclear microphonic was observed in these groups at lower frequencies, the biological significance of which was considered to be doubtful since it may have been an artefact of the technique employed. Overall, since styrene alone did not produce any effects and the combined exposure did not result in any auditory impairment over and above that induced by noise alone this part of the study provided no evidence for any interaction.

The next part of the study was similar in design to the previous part with groups receiving either 500 or 1200 ppm styrene simultaneously with noise for seven hours, noise alone or no treatment; there was no styrene only group. Auditory function was assessed at one week post-exposure. Again, there was no indication that styrene combined with noise produced any enhancement of effects compared to noise alone.

The acute inhalation toxicity of styrene in the mouse is more variable than in other rodent species. An inhalation LC_{50} value of 4930 ppm for a 2-hour exposure has been reported in an unnamed mouse strain (Shugaev and Yaroslavi, 1969). However, in $B6C3F_1$ mice, deaths occurred about 12 hours after one 6-hour exposure to 500 ppm or 2 exposures to 250 ppm (Morgan *et al*, 1993a). In male $B6C3F_1$ mice, 8 of 27 died at 500 ppm and 11 of 25 died at 250 ppm. Fewer deaths occurred in female $B6C3F_1$ mice. Centrilobular coagulative necrosis of the liver was found in the decedents. There were no deaths or effects on the liver at 125 ppm. These data are in contrast to results indicating little hepatic toxicity in the rat and guinea pig studies. An acute inhalation study comparing the extent of depletion of hepatic GSH levels following exposure to styrene produced results indicating much more pronounced depletion in mice, compared with rats (Filser *et al*, 1999). It is attractive to link the relative sensitivity of mouse to the greater capacity in the mouse to generate styrene oxide at high exposure levels. However, in one study in different strains of mice, blood levels of this metabolite did not correlate with the extent of hepatotoxicity (changes in liver weight) in different strains of mice (Morgan *et a*l, 1993c).

A series of studies has been conducted in the $B6C3F_1$ mouse investigating potential hepatotoxicity following single and repeated exposures to styrene (Mahler *et al*, 1999). The repeated exposure data are reported in section 4.1.2.6.1. In animals exposed to a single exposure of 500 ppm styrene for 6 hours clear signs of liver damage (liver weights increased by 26%, centrilobular hepatocellular necrosis, increased hepatocyte cell proliferation) and associated deaths due to liver damage were observed at day 5 post-exposure. At day 14 post-exposure all parameters were similar to controls.

In a recent study investigating the pulmonary toxicity of styrene in mice, groups of 10 male CD-1 mice were exposed whole-body to 0,40 or 160 ppm styrene (99% purity) for 2, 4 or 6

hours (Green, 2000b). Animals were killed immediately post-exposure. Additional groups of animals were maintained and sacrificed 18 hours after the 6 hour exposure period. The lungs were excised and examined macro and microscopically. No clinical signs of toxicity were observed in any of the animals during exposure or during the 18-hour recovery period. At microscopy, a dose-related and treatment-related increase in the incidence of loss of apical cytoplasm from the non-ciliated cells of the bronchioles (Clara cells) was observed in animals exposed for 4 and 6 hours to 40 ppm (1/10 and 4/10, respectively) and 160 ppm (7/10 and 8/10, respectively). Following the 18-hour recovery period this finding was absent in animals at both exposure levels. No findings of this type were observed in control animals or in animals exposed for 2 hours only. No adverse findings in the bronchi or alveoli of any animal were observed.

Oral

Oral styrene is of low acute oral toxicity to rats and hamsters following single oral dosing. A total of 59 Wistar rats (number of animals/group not specified) were administered different single doses of styrene (range not specified in olive oil by oral gavage (Spencer *et al*, 1942 and Wolf *et al*, 1956). All animals administered 160 mg/kg survived for the 2-week observation period post-dosing, whereas al animals administered 8000 mg/kg died within the observation period. No details of signs of toxicity or histopathological findings were reported. An oral LD_{50} value in the rat was determined from this study to be approximately 5000 mg/kg.

A study is available which investigates the hepatotoxicity of styrene in hamsters (Parkki, 1978). Groups of 23 male Syrian hamsters were administered 0, 450 or 600 mg/kg styrene in corn oil by oral gavage. At 24 hours post-exposure animals were sacrificed and serum alkaline phosphatase (AP), alanine aminotransferase (ALT) and epoxide hydrolase activity were measured. Livers were removed and the glutathione (GSH) content was determined. No treatment-related effects on the parameters investigated were observed in animals receiving 450 mg/kg compared with controls. In animals administered 600 mg/kg, 3/23 animals died within 24 hours of dosing. The GSH content of the livers of animals receiving 600 mg/kg was statistically significantly decreased to 15% of control levels and serum ALT levels were statistically significantly increased by 10-fold compared with controls.

Dermal

No studies on acute dermal toxicity have been reported. Based on the available toxicokinetic, toxicodynamic and physiocochemical data it is predicted that systemic toxicity via this route of exposure would be low.

4.1.2.2.2 Studies in humans

Studies in volunteers

Two volunteers exposed to 800 ppm styrene for up to 3 hours experienced irritation of the eyes and nose immediately after the start of the exposure period (Carpenter *et a*l, 1944). This was followed by symptoms of listlessness, sleepiness, lack of muscular coordination, weakness, disturbances in equilibrium and depression. These symptoms indicate the CNS depressant or pre-narcotic effect of styrene. No unexposed control individuals were assessed in the study.

Six studies are available in which naïve volunteers were given a single inhalation exposure to styrene and subjected to neurobehavioural (psychological) tests. These tests were generally not specific but rather addressed mixed nervous system functions.

In one study 1-5 subjects were exposed to 50, 117, 216 and 376 ppm styrene for one hour (or 2 hours in the case of 117 ppm in an exposure chamber (Stewart *et al*, 1968). During the exposure period they were subjected to a range of neurobehavioural tests designed to measure manual dexterity (Crawford tests) and coordination (Flannagan test). Impaired performance, 10-30% below pre-exposure values, was noted in all the psychomotor tests at 376 ppm but no significant effects were observed at 216 ppm or below. In the same series of studies, 6 volunteers were exposed to 100 ppm of styrene for 7 hours, with a 30-minute break half-way through the exposure period. These individuals were subjected to the same range of neurobehavioural tests as before, at various times during the exposure period. No significant effects were reported.

Other workers have reported mild impairment in neurobehavioural test performance in volunteers exposed to similar styrene levels. In one study, 12 volunteers were exposed to 50, 150, 250 and 350 ppm styrene for 30 minutes, the exposures running consecutively (Gamberale and Hultegren, 1974). Exposure was by way of a mouthpiece, so as to prevent symptoms of eye and nose irritation, but was not possible to prevent the subjects being aware of exposure to styrene at the higher concentrations, due to the strong metallic taste of the vapour. The men were subjected to a range of neurobehavioural tests during the exposure period: these were broadly designed to measure perceptual speed, reaction time, manual dexterity and psychological state. The only significant effect noted was a slight statistically significant impairment in reaction time at 350 ppm only.

In another study, 2-3 volunteers were subjected to neurobehavioural tests whilst exposed to 50, 100 or 200 ppm styrene for 1.5 hours (Oltramare *et al*, 1974). The tests consisted of reaction time to a simple visual stimulus, reaction time to visual and acoustic stimuli and reaction time to multiple stimuli (test of diffuse attention). Some slight reduction (20-30%) in response to visual stimuli, and also to visual/acoustic stimuli was noted at all concentrations investigated. However, there was no dose-response trend, suggesting that these changes were perhaps not related to styrene exposure. Slight reduction in performance (10%) was noted in the diffuse attention test, at 200 ppm only.

Pierce *et al.* (1998) investigated the effects of 4 different styrene exposure regimes on neuro-physiological and neuro-behavioural tests in normal, healthy volunteers. Four male individuals aged 26-30 with no history of exposure to solvents were selected. These were non-smokers, were not on medication and did not drink more than 18 g of alcohol per day. Subjects were exposed to styrene in a 13.8 m^3 chamber. Four exposure regimes were used: day 1 - three sequential 100-minute periods of 15, 32.5 and 50 ppm; day2 (next day) – three sequential 100-minute periods of 50, 75 and 99 ppm; day 3 (several weeks later) – fluctuating exposure levels between 10 and 150 ppm for 4 hours; day 4 (next day) – a constant exposure of about 10 ppm followed by a highly variable auto-correlated exposure of 5-200 ppm for 4 hours. Three subjects (A, B and C) participated in the day 1/day 2 exposures, and two subjects (A and D) participated in the day 3/day 4 exposures. Blood styrene levels were measured once before, six times during, and four times after each exposure regimen. The ‘2’ and ‘7’ digit recognition test, which consists of identifying 2s and 7s from a series of random digits, was performed on each subject on days 1 and 2 of exposure to styrene prior to exposure and after 35 minutes of exposure. The latency and amplitude of a P300 auditory evoked potential (an event-related brain wave with a latency of approximately 300 ms in normal subjects considered to be an indicator of subtle cognitive effects) was measured using scalp electrodes following an auditory stimulus. The latency was measured as the time between tone and peak of the P300 wave, and the amplitude was measured from the preceding negative peak to the P300 peak. The P300 potential test was

administered on each subject on days 1 and 2 of exposure to styrene prior to exposure and at the end of each 100-minute exposure periods.

No changes in the P300 test amplitude or latency were observed with exposure to styrene when comparing the post-exposure values with the pre-exposure values. Scores for the '2' and '7' digit recognition test improved between days 1 and 2, but were considered to be due to increased familiarity with the test. These experimental data obtained from four volunteers provide no evidence that short exposures to concentrations up to 150 ppm styrene have adverse effects on the nervous system.

Nine groups of 4-5 healthy male volunteers for a total of 42 subjects, aged 20-50 years, with no previous exposure to styrene or other neurotoxic agents (although 5 had had occasional exposure to paint solvents) were exposed in a 18 m^3 chamber to 5 different styrene exposure regimes to investigate potential acute neurotoxic effects (Ska et al., 2003). All subjects were self-reported 'social' consumers of alcohol. Exposure sessions (a total of 6 hours per day) took place once every 2 weeks for each group. Each volunteer was successively exposed to 5 exposure regimes each of 6 hours duration: a) constant at 106 mg/m^3 (25 ppm); b) variable with a mean concentration of 106 mg/m^3 but with four 15-min peaks up to 213 mg/m^3 (50 ppm); c) constant at 213 mg/m^3; d) variable with a mean concentration of 213 mg/m^3 but with peaks up to 426 mg/m^3 (100 ppm); e) constant at the low level of 5 mg/m^3 – this was used as a 'null exposure' for physio-neurological effects but still with a detectable odour. On each experimental day the subjects were tested pre- and post-exposure. Three kinds of tests were used: sensory tests (colour vision assessed by a Lanthony D-15 desaturated panel, a vision contrast test, and an olfactory threshold test); neurobehavioural tests (simple reaction time, colour-word stress test, symbol digit matching test, digit span memory test, and continuous tracking test); and a questionnaire to assess mood and symptoms (local irritation or CNS effects).

Only the results obtained from those subjects completing all exposures (24 of the initial 42) were included in the statistical analysis. No significant effect of exposure on the sensory tests was noted. In the neurobehavioural tests subjects had a faster reaction time on the colour-word stress test post-exposure compared to the pre-exposure values regardless of exposure, although accuracy remained unchanged; subjects had a faster response time on the symbol digit matching test after exposure compared with before, and as they progressed through sessions; and subjects remembered a higher number of digits on the digit span memory test as they progressed through sessions. These results are considered to be an expression of the increased familiarity of the volunteers with the tests administered. No other significant effect was noted. No significant effect of exposure on mood or symptoms was reported. It can be concluded that short-term exposures up to 50 ppm for 6 hours with peaks up to 100 ppm did not induce acute neurotoxic effects in 24 volunteers.

Groups of 8 healthy male volunteers (aged 22-36 years) without previous occupational exposure to solvents were exposed, in a blinded manner, to 0.5 or 20 ppm styrene in a chamber for 3 hours (Seeber *et al*, 2004). A second investigation involved additional groups of 12 male volunteers exposed to 0.5 or 20 ppm at a constant level for 4 hours and to an additional changing exposure between 0.5 and 40 ppm (mean 14 ppm) for other 4 hours. To achieve a mean concentration of 14 ppm there were two peak exposures of 40 ppm lasting 30 minutes each and two periods of 50 and 70 minutes at 0.5 ppm as well as the transition times between peaks and troughs. Investigations included simple visuomotor reaction time, choice reaction, cancellation test d2 (for attention), Sternberg memory scanning (for short-term memory) and changes in the state of well-being. The acute symptoms ("discomfort", "irritation", "tiredness", "breathing problems") were scored on a scale of 0-5 ("not at all" to "very strong"). Other ratings of well-being ("tension", "tiredness", "complaints", "annoyance") were scored on a score of 1-7 ("not annoying " to "very annoying"). Before styrene exposure was administered, these tests were assayed for validity and reliability. Also before exposure,

volunteers were screened to exclude those with a pre-existing medical condition that may have influenced results. Those left in the study were trained in each of the tests.

The authors indicated that the mean simple reaction time and choice reaction time improved markedly when exposure and testing were conducted in the afternoon; this was taken to be an effect related to the time of day rather than a substance-related effect. There were no meaningful differences in the mean scores for acute symptoms obtained for groups of people exposed to 0.5 ppm or 20 ppm for 4 hours or for those exposed also to two peaks of up to 40 ppm. Overall, there were no exposure-related changes in any of the performance tests, symptoms or changes in the state of well-being amongst these volunteers exposed to up to 20 ppm continuously for 4 hours and amongst those additionally exposed to two peaks of up to 40 ppm during an additional 4-hour period (average 14 ppm).

Overall, considering the six neurobehavioural testing studies together, the results have been variable. There has been no convincing evidence of an effect on test performance with exposures in the range 0.5 – 150 ppm.

The potential for a single exposure to styrene to produce vestibulo-oculomotor disturbances was investigated in a group of 5 male and 5 female volunteers (Odkvist *et al*, 1982). There were no relevant disease in the medical histories and alcohol was not ingested in the 24 hours before exposure. All individuals were non-smokers and had no history of eye or auditory disease. Exposure was for one hour and was to a concentration of between 87 and 139 ppm styrene (concentrations brethed in were constantly monitored). During exposure, the subjects performed “slight” physical work on a bicycle. Individuals underwent a sinusoidal swing test, an optovestibular test and tests for visual suppression and optokinetics, as well as a slow pursuit movement test. For comparison purposes the tests were repeated on the individuals one week prior to and one week after styrene exposure, during which time the individuals inhaled clean air. No abnormalities were detected on electronystagmographic examination before exposure. The only effects of exposure on test results were disturbance of the visual suppression system and enhanced speed of the saccade (quick movement of the eye in response to a light source), in 3/10 exposed individuals compared with when these individuals breathed clean air only. No clear interpretation can be placed on these results.

A case study has been reported of a 36-year old man who twice developed neurological symptoms after using a home canoe construction kit (MacFarlane *et al*, 1984). Styrene vapour exposure was known to be associated with use of the kit and in this case work had been carried out in an enclosed space for 4-5 hours. The man became dizzy and lost consciousness on standing. Also, conjunctivitis, slurred speech, lateral and upward gaze nystagmus and limb ataxia were noted. The styrene exposures associated with this case are not known. Also, it is not reported what other constituents were present in the kit. Hence a possible contribution from other substances cannot be dismissed.

4.1.2.2.3 Summary of acute toxicity

In humans there is some acute inhalation toxicity information available indicating effects of styrene on the central nervous system (CNS) function. From the studies that have been reported there has been no convincing evidence of an effect on neurobehavioural test performance with exposures in the range 0.5 – 150 ppm. However, some impairment in test performance (reaction time, manual dexterity and coordination) appeared with exposures of 200, 350 and 376 ppm for periods of 30 – 90 minutes. Higher concentrations (800 ppm in one study) have produced signs and symptoms of pronounced CNS depression. No other acute toxicity information is available from human studies.

In rats and guinea pigs styrene is of moderate-low acute toxicity via the inhalation and oral routes. Two inhalation studies in rats have reported a 4-hour LC_{50} of 2770 ppm (11.8 mg/l) and “some deaths” at 2149 ppm (9.1mg/l) for up to 40 hours. An oral LD_{50} of approximately 5000 mg/kg has been reported in the rats. In contrast, mice (at least some strains of mice) are much more sensitive to a single exposure to styrene, with cellular damage in the respiratory epithelium at 40 ppm (the lowest concentration tested) and fatal hepatocellular damage at 250 ppm and above in acute inhalation studies. The most likely explanation for this species difference is the greater potential for build-up of the reactive styrene oxide metabolite in mouse, compared to rat or human. An acute oral study in hamsters also indicates styrene-induced hepatoxicity at 600 mg/kg (but not at 450 mg/kg). No acute dermal toxicity studies have been performed in experimental animals, but one would predict low acute toxicity.

In view of the fact that humans (volunteers and workers) have been exposed without serious effects to acute exposure conditions that have proved toxic and even lethal to more sensitive mouse strains, and considering the known toxicokinetic difference between the mouse and the human in the activation/deactivation of styrene, the mouse is considered to be a poor and unreliable model for the acute toxicity of styrene in humans.

Therefore, for the purposes of risk characterization, information from the human volunteer studies will be used. The most useful reference point in relation to short-term single exposure is the observation that no CNS depression was seen in humans exposed to 100 ppm for 7 hours and that some minor impairment in neurobehavioural test performance was observed at about 200 ppm for 1 hour.

4.1.2.3 Irritation

4.1.2.3.1 Skin irritation

Studies in animals

No conventional skin irritation studies using a single application of styrene liquid are available.

A single application of undiluted styrene to the rabbit ear is reported to have caused no appreciable reaction whereas two occlusive applications of undiluted styrene to the abdomen of the rabbit are reported to have produced signs of a “marked irritation” reaction with denaturation (Spencer *et al*, 1942). In a study reported by the same authors, the repeated application of undiluted styrene to rabbit ears, 20 times over four weeks, produced “moderate irritation”, with blistering and hair loss. Wolf *et al* (1956) reported moderate “definite erythema” with “slight necrosis” (development of a thin layer of devitalised tissue which resulted in exfoliation) on the surface skin of albino rabbits following repeated occlusive skin application of 10-20 doses of undiluted styrene over 2-4 weeks to the ear and abdomen. No other details are available.

Studies in humans

No reports of skin irritation observed in humans exposed to styrene are available. In a study in which 9 volunteers immersed a hand into undiluted styrene for up to 30 minutes there is no mention of any signs of skin irritation (Berode *et al*, 1985).

Overall, the quality of information on the skin irritation potential of liquid styrene is poor, particularly in relation to the consequences of a single application. The available data suggest that a single exposure is not significantly irritating, but that repeated exposure does cause irritation.

4.1.2.3.2 Eye irritation

Studies in animals

In a study by Wolf *et al* (1956), two drops of undiluted liquid styrene were applied to the right eye of an unspecified number of albino rabbits. Visual inspection for irritation and corneal injury (both internal and external) was made on the treated eyes at 3 minutes, 1 hour, and 1, 2 and 7 days post-instillation. Moderate conjunctival irritation (inflammation and slight swelling of the eyelids) and slight, transient corneal injury (perceptible superficial necrosis involving <50% of the lens) were reported.

In an acute inhalation exposure study (see section 4.1.2.2.1) evidence of eye irritation, described as lachrymation with rubbing and scratching of the eyes, was reported in rats and guinea pigs immediately following the onset of exposure to 1449 ppm styrene and above (Spencer et al, 1942). The severity of the response increased with higher exposure concentrations.

Some ocular irritation potential for styrene has also been demonstrated in an *in vitro* assay using bovine lens cultures (Herbet and Sivak, 1996).

Studies in humans

In a volunteer study 9 subjects were exposed to 50-375 ppm styrene for one hour (Stewart *et al*, 1969). Nine laboratory personnel served as control subjects. At 375 ppm, 4 exposed subjects reported “mild eye irritation” and all reported “nasal irritation”; one subject experienced a burning sensation on the face. No subjective reports of eye irritation were reported at any of the lower exposure concentrations (50, 100 or 216 ppm) or by controls. In the same series of studies, 6 volunteers were exposed to 100 ppm styrene for 7 hours, with a 30 minute break half-way through the exposure period: following exposure all reported very mild and transient eye “irritation”. Eye irritation was reported in volunteers immediately following exposure to 800 ppm styrene. In all of the above studies no clinical examinations were reported and the subjective assessments were made by the volunteers themselves.

In a study by Wolf *et al* (1956), a group of human volunteers (number not specified) was exposed to a wide range of styrene concentrations. Reactions with respect to odour, eye irritation and nasal irritation were noted by the individuals themselves from the onset of exposure. “Strong” eye irritation was reported at exposure concentrations of $\geq$ 600 ppm. Odour but not irritation was reported at 60 ppm and no odour or irritation was reported at concentrations of < 10 ppm.

Eye irritation was reported in 2 volunteers immediately upon the onset of exposure to 800 ppm styrene (Carpenter *et al*, 1944).

Overall, the available evidence suggests that liquid styrene can produce eye irritation. Exposure to airborne styrene vapour can also cause eye irritation. Concentrations of 375 ppm and above are clearly irritating. Exposures of up to approximately 216 ppm for one hour

were without effect. Although slight “irritation” was reported by subjects following exposure to 100 ppm styrene for 7 hours, absence of effects in the same study at 216 ppm for 1 hour exposure suggests that the effect with longer exposure was not primary irritation, but perhaps eye dryness or some similar sensation.

4.1.2.3.3 Respiratory tract irritation

Studies in animals

An acute inhalation toxicity study reported signs of marked irritation of the nasal mucosa (nasal discharge and nasal scratching) in rats and guinea pigs immediately following onset of exposure to 1449 ppm (the lowest concentration tested) (Spencer *et al*, 1942, see section 4.1.2.2.2). The severity of effects increased with higher exposure concentrations.

In a review by Bos *et al* (1992), 3 studies reporting RD50 values for styrene are cited. The studies were all Alarie assays conducted in Swiss mice. In one (Alarie, 1973), an RD_{50} value of 157 ppm (95% CI 135-178) was reported following an exposure time of 3 minutes. In another (Alarie, 1981), an RD_{50} value of 980 ppm (95% CI 826-1297) was reported following an exposure time of 10 minutes, and in the third study (De Ceaurriz *et al*, 1981) and RD_{50} value of 586 ppm (95% CI not presented) was reported following an exposure time of 5 minutes.

Studies in humans

In a volunteer study 9 subjects were exposed to 50-375 ppm for one hour (Stewart *et al*, 1968). Nine laboratory personnel served as controls. One exposed subject reported “nasal irritation” after 20 minutes exposure to 216 ppm. At 375 ppm, 4 exposed subjects reported “nasal irritation”; one subject experienced a burning sensation on the face. There were no subjective reports of nasal irritation at any of the other exposure concentrations or in controls. In the same study no adverse effects were reported by 10 subjects exposed to up to 100 ppm styrene for 1 hour or for 2 x 3.5 hours (with a 30 minute interval between exposures).

In a study by Wolf *et al* (1956), a group of human volunteers (number not specified) was exposed to a wide range of styrene concentrations (exact range not reported). Reactions with respect to odour perception, eye irritation and nasal irritation were noted by the individuals from the onset of exposure. “Severe” nasal irritation was reported at exposure concentrations of $\geq$ 600 ppm. Odour but no irritation was reported at 60 ppm and no odour was reported at concentrations of < 10 ppm.

In another study 2 volunteers exposed to 800 ppm for up to 4 hours experienced irritation of the nose with increased nasal mucous secretion immediately after the start of the exposure period (Carpenter *et al*, 1944).

Overall, it is clear that exposures to airborne styrene can cause respiratory tract (nasal) irritation. No effects were seen at 100 ppm for 1 hour or 7 hours and only one subject out of nine reported nasal irritation at 216 ppm for 1 hour, suggestting little or no significant irritation at this concentration. Nasal irritation was more evident at 375 ppm for 1 hour and above, in several studies.

4.1.2.3.4 Summary of irritation

The quality of information on the skin irritation potential of liquid styrene is poor, particularly in relation to the consequences of a single application. The available data suggest that a single exposure is not significantly irritating, but that repeated exposure does cause irritation.

The available evidence suggests that liquid styrene can produce eye irritation. Exposure to airborne styrene vapour can also cause eye irritation. Concentrations of 375 ppm and above are clearly irritating. Exposures of up to approximately 216 ppm for one hour were without effect. Although slight eye "irritation" was reported by subjects following exposure to 100 ppm styrene for 7 hours, absence of effects in the same study at 200 ppm for 1 hour exposure suggests that the effect with longer exposure was not primary irritation, but perhaps eye dryness or some similar sensation. NOAEC values of 216 ppm for 1 hour and of 100 ppm for 7 hours are therefore identified for risk characterisation purposes.

It is clear that exposures to airborne styrene can cause respiratory tract (nasal) irritation. No effects were seen at 100 ppm for 1 hour or 7 hours and only one subject out of nine reported nasal irritation at 216 ppm for 1 hour, suggesting little or no significant irritation at this concentration. Nasal irritation was more evident at 375 ppm for 1 hour and above, in several studies. NOAEC values of 216 ppm for 1 hour and of 100 ppm for 7 hours are therefore identified for risk characterisation purposes.

4.1.2.4 Corrosivity

The studies in animals and humans reported in 4.1.2.3 indicate that styrene is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Skin sensitisation

Studies in animals

Two reports of guinea pig maximization tests are available. However, unfortunately neither is well reported. In one study, 15 animals were exposed to styrene intradermally (a 10% concentration) and topically (a 20% concentration) at induction and topically to 2% at challenge (Sjoborg *et al*, 1982). Apparently no animals were sensitised.

In the second study, a number of chemicals including styrene were tested using the protocol of Magnusson and Kligman (1969) (Senma *et al*, 1978). Induction concentrations were 5% by both topical and intradermal routes and the topical challenge concentration was 1%. There is a contradiction in the reporting of the results; the incidence of skin reactions was either 11% (1/9) or 33% (3/9) in the 9 animals scored. Because of this ambiguity, no conclusions can be drawn from this particular study, when considered in isolation.

Studies in humans

There is vast human experience of skin contact with styrene over many years but only one case of skin sensitisation has been claimed. It is reported that a patient developed dermatitis after exposure to styrene in the use of a plastic repair kit for a second time (Sjoborg *et al,*

1982). The plastic was unsaturated polyester with styrene and benzoyl peroxide hardener. Patch testing with an internationally agreed series and with polymerisation inhibitors which are added to styrene gave negative reactions. However, there was a positive reaction with styrene.

Overall, in light of the extensive potential for exposure and the widespread use of styrene the absence of reports of styrene-induced skin sensitisation indicates that styrene has no significant potential to cause this effect.

4.1.2.5.2 Respiratory tract sensitisation

Studies in animals

There is no information available in animals relating to respiratory sensitization.

Studies in humans

There is a vast amount of data pertaining to human occupational, and some consumer, inhalation exposure to styrene. Against this background, only two cases of asthma allegedly associated with styrene exposure have been recorded.

One case of asthma associated with occupational exposure to styrene during figreglass mould work has been reported (Hayes, 1991). The polyester resin was mixed with an accelerator containing cobalt octoate and organic peroxide catalyst. The individual had no previous history of asthma or hayfever. The patient was tested in several single blind inhalation tests on separate days using a styrene brush painting task with and without cobalt octoate. A dual asthmatic response and increased responsiveness to inhaled histamine were observed in both instances, although the reaction was more severe when cobalt octoate was present. Self-recorded peak expiratory flow deteriorated in work periods and improved away from work. The presence of cobalt, a known respiratory sensitizer, confounds interpretation of this case.

Another case of a person occupationally exposed to styrene (job description not reported) and assessed using bronchial challenge was reported in abstract form only (Moscato *et al*, 1990). Symptoms of cough, breathlessness and wheezing were experienced, which disappeared when the patient was away from styrene exposure at work. The person had no history of atopy. Bronchial challenge with a concentration of 15 ppm styrene apparently provoked a dual asthmatic reaction assessed by measuring FEV_1. No further details were available in the abstract. The lack of detail surrounding this case prevents any definite conclusions being drawn.

Overall, considering the vast amount of human occupational and some consumer inhalation exposure to styrene, the fact that the literature contains only two case reports of asthma, each of which have unconvincing aspects to it, suggests that styrene does not possess significant potential to cause respiratory sensitization.

4.1.2.5.3 Summary of sensitisation

The reporting of the available animal skin sensitization data is inadequate, precluding a clear conclusion being drawn from the studies themselves. However, given that widespread

exposure to styrene has led to only one reported possible case of skin sensitization, this extensive human experience indicates that styrene is not a significant skin sensitizer and negates the need for any further animal testing with respect to this endpoint. Similarly, there has been extensive inhalation exposure in humans which has resulted in only two case reports of asthma, each of which has unconvincing aspects to it. This suggests that styrene has no significant asthmagenic potential.

4.1.2.6 Repeated dose toxicity.

4.1.2.6.1 Studies in animals

The repeated exposure toxicity of styrene in animals has been extensively investigated, although some of the available studies have been limited to an investigation of certain aspects of toxicity such as the effects on the liver or nervous system. This section is organised firstly in terms of exposure route, and then subdivided in relation to the species and nature of the study.

Inhalation

Rats

General studies in rats investigating a range of toxicological endpoints

In a well conducted combined chronic toxicity/carcinogenicity study, groups of 70 male and 70 female Sprague Dawley rats were exposed whole-body to 0, 50, 200, 500 or 1000 ppm styrene monomer vapour (97.7 to 99.5% purity) for 6 hours/day, 5 days/week for 104 weeks (Hardy *et al*, 1996 and Cruzan *et al*, 1998). Ten animals per sex per group were sacrificed at week 52 for interim analyses. The remaining 60 animals/sex/group were maintained for the remainder of the study duration (see section 4.1.2.8.1 fo study details).

The only clinical sign of toxicity observed was increased salivation with restlessness, followed by hunched posture, in animals exposed to 500 and 1000 ppm, from week 19 and 1, respectively, for the initial 10 – 30 minutes post-exposure.

Relative to controls, body weight gain was unchanged in males and females at 50 and 200 ppm. In males at 500 and 1000 ppm, body weight gain was reduced by 12 and 15%, respectively between weeks 0 and 53, and by 3 and 11% at week 104. In females at 500 and 1000 ppm, body weight gain was reduced by 22 and 25% respectively between weeks 0 and 53, and by 34 and 23% at week 104. Food consumption was similar across all groups. Water consumption was increased in a dose-responsive manner in all animals by 6 to 60% compared with controls, with females consuming more water than males.

Haematological, clinical chemistry and urine analyses revealed no treatment-related changes in any of the animals examined. Ophthalmoscopic examination did not reveal any treatment-related changes in any of the groups.

At both the 52 and 104 week necropsies, absolute and relative organ weights of treated animals were comparable with controls and no adverse macroscopic pathological findings were observed. Microscopic examination at weeks 52 and 104 revealed treatment-related changes in the nasal passages of exposed animals from all styrene-exposed groups. The changes were confined to the olfactory epithelium and included focal disorganization with rosette formation, focal hyperplasia of the basal cells, atrophy/degeneration/erosion and

prominent Bowman's glandular elements. In all groups the lesions were focal and of "minimal" severity but there was a dose-related increase in the incidence and distribution of these changes, with incidences in males of 31, 48, 62 and 73% at 50, 200, 500 and 1000 ppm, respectively, compared with 0 in controls, and incidences in females of 10, 24, 37 and 48% at 50, 200, 500 and 1000 ppm, respectively, again compared with 0 in controls.

No treatment-related thyroid effects were observed in animals in exposed to 50, 200 or 500 ppm at week 52 or any animals at week 104. However at week 52, in both males and females exposed to 1000 ppm, minimal follicular epithelial hypertrophy of the thyroids was observed. No thyroid changes were observed in controls. In the absence of effects at 104 weeks, it is probable that these thyroid effects were not treatment-related. No other non-neoplastic effects were observed. Neoplastic changes are reported in section 4.1.2.8.1.

Overall, these data indicate that in the rat the nasal passages are a principal target tissue for styrene toxicity following exposure via the inhalation route under the conditions of this study, with mild inflammatory effects being observed in animals from all exposure groups (≥ 50 ppm) at both 52 and 104 weeks. Based on the histopathological changes in the nasal passages a LOAEC for local effects of 50 ppm is evident from this study. No signs of systematic toxicity were observed at exposure concentrations up to 200 ppm, above which various clinical signs and reduced body weight gain were reported.

In a carcinogenicity study, groups of 85 male and 85 female Sprague Dawley rats, were exposed whole-body to 0, 600 or 1000/1200 ppm styrene for 6 hours/day, 5 days/week (Jersey *et al,* 1978). The high exposure group was exposed to 1200 ppm for the first two months but this produced marked signs of toxicity (narcosis, anaesthesia and death in three animals), and was reduced to about 1000 ppm for the remainder of the study. The males were exposed for about 18 months and the females for about 21 months. The study was terminated at 24 months (see section 4.1.2.8.1 for further study details). At necropsy, relative to the control group, the amount of adipose tissue was decreased and liver, kidney and heart organ weights were reduced in the 1000 ppm group. Microscopically, "very slight" histopathological changes in the liver (decreased amount of micro-vesiculature or vacuolation of hepatocytes), suggestive of decreased glycogen and fat, were observed at 600 and 1000 ppm. There were no treatment-related effects on the testes or other organs examined. It is not stated whether or not the nasal passages and the upper respiratory tract were examined in this study, but it appears unlikely, given the findings at this site in other repeated inhalation studies in rats.

A well-conducted 13-week rat study is available (Hardy *et al,* 1993 and Cruzan *et al,* 1997). Ten male and 10 female Sprague-Dawley rats were exposed to 0, 200, 500, 1000 or 1500 ppm styrene vapour for 6 hours/day, 5 days/week for 13 weeks. Bodyweight, food and water consumption, clinical chemistry and haematology were evaluated at 4 and 13 weeks. Organ weights, and macroscopic and microscopic pathology in a wide range of organs and tissues were assessed at 13 weeks. A satellite group of 15 males was exposed using the same regimen for 2, 5 or 13 weeks, at which time lung and liver cell proliferation was determined by 5-bromo-deoxyuridine (BrdU) labelling.

No treatment-related deaths occurred. Signs of slight local irritation such as closing of the eyes were noted at 200 ppm during exposure; signs at higher concentrations indicated dose-related increasing severity of irritation. Body weight gain was reduced to 81% of control values in males at 1500 ppm and food consumption was also reduced in this group. There were dose-related increases in water consumption in groups exposed to 1000 and 1500 ppm. The only other exposure-related effects were histopathological changes in the olfactory epithelium; focal disorganisation or hyperplasia of basal cells, single cell necrosis or cell loss were recorded. These changes were observed at 500 -1500 ppm, the incidence and severity of the microscopic effects being dose-related. BrdU labelling showed no evidence of

increased cell proliferation in the rat liver cells or in the cells of the bronchiolar or alveolar regions of the rat lung, at any time point investigated.

The effect of repeated inhalation exposure to styrene on Wistar rats was investigated in an early study by Spencer *et al,* (1942). Groups of 28 and 50 rats, respectively, were exposed to 0, 1449 or 2139 ppm styrene for 7-8 hours/day 5 days/week for 21 weeks. During exposure, regular body weight checks and observations of clinical toxicity were made. Frequent blood samples from representative animals were also taken for haematological analysis (timings of sampling not reported). At the end of the exposure period gross and microscopic examination of an extensive range of tissues and organs was conducted.

No treatment-related deaths occurred in any animals. In animals exposed to 1449 ppm styrene, no changes in body weight gain compared with controls were observed. However body weight gains were reportedly reduced in animals at 2139 ppm styrene compared with controls (not quantified). Signs of eye and nasal irritation were observed in animals of both exposure groups. Animals exposed to 2139 ppm styrene also appeared listless, dirty and unkempt. No changes in haematology, organ weights, gross or microscopic pathology were observed in animals of either exposure group. However, it would appear that the nasal tissues were not examined.

In a briefly reported study (abstract only available), groups of F344 rats (number per group not specified) were exposed to 0, or 125 to 1500 ppm styrene for 90 days (exposure regimen not detailed) (Roycroft *et al,* 1992). No treatment-related deaths were reported and no clinical signs of toxicity were observed. Body weight gain was reportedly reduced (not quantified) in rats at 1500 ppm. Relative liver weights were apparently increased (not quantified) in rats at the two exposures. Haematological and biochemical analysis apparently revealed no treatment-related variations in rats of either sex in any of the exposure groups. Histopathology revealed degeneration and necrosis of the nasal olfactory epithelium and goblet cell hypertrophy of the nasopharyngeal duct, apparently observed in animals of all styrene-exposed groups. No information on the incidence of severity of these findings was reported.

A study by Ohashi *et al,* (1985), investigating the respiratory toxicity of styrene, is cited in the review by Bond (1989). In the study, groups of rats (strain and number not specified) were exposed to up to 800 ppm styrene for 4 hours/day for 8 weeks. Animals were sacrificed and examined histopathologically 3 weeks after the last exposure. It is reported that changes in the nasal mucosa occurred at 50 ppm and above, including vacuolation of epithelial cells, nuclear pyknosis, and ‘fall-off’ of epithelial cells. The incidence and severity of the findings is not given. It is reported that in all cases damage was more severe in the upper respiratory tract compared with the lower respiratory tract.

In a follow-up study by the same workers, Ohashi *et al,* (1986), also cited in the review by Bond (1989), rats (strain and number not specified) were exposed to 150 or 1000 ppm styrene for 4 hours/day, 5 days/week for 3 weeks. Animals were sacrificed and examined histopathologically at up to 12 weeks after the last exposure. At day 1 after the end of the exposure period it is reported that there was a dose-dependent decrease in tracheal and nasal ciliary activity, and that ciliastasis was observed in animals exposed to 1000 ppm. At week 12 post-exposure, in animals exposed to 150 ppm, ciliary activity and nasal tracheal mucosal morphology were comparable with controls. In animals exposed to 1000 ppm, ciliary activity was only 50 to 75% of that of control values, and nasal mucosal morphology remained abnormal, whereas tracheal mucosal morphology was comparable with controls.

In one investigation in which rats were exposed to 300 ppm styrene for 6 hours/day, 5 days/week for 2 weeks or more, histological evidence of hepatotoxicity (hydropic degeneration, steatosis and congestion) was claimed (Vainio et al, 1979). However, these

results are unlikely to represent styrene-related liver toxicity, as there are other studies in which no or very mild effects on liver histopathology were observed at higher exposure concentrations (200-1500 ppm) – see above. No evidence of any lung or kidney lesions was noted after up to 11 weeks, when the experiment was terminated. The activity of certain enzyme systems involved in xenobiotic metabolism was increased in the liver and kidneys, but not in the lungs. Other studies have also shown increases in the activity of these enzyme systems in the liver and kidneys, but not the lungs, following exposure of rats to 450 ppm 8 hours/day for 7 days (Sandell et al, 1978).

An inhalation study specifically designed to investigate mechanistically the putative styrene-induced reproductive impairment has been reported (Jarry et al., 2004). The study looked for any potential correlations between the lutenizing hormone (LH) and testosterone (T) serum levels in styrene-exposed rats with the brain concentrations of the neurotransmitters gamma-amino butyric acid (GABA) and glutamate (GLU), which control the release of gonadotrophin-releasing hormone (GnRH) from the mediobasal hypothalamus (MBH), which in turn controls the release of LH and T. Groups of ten male Wistar rats were exposed to 0, 150, 500 and 1500 ppm (0, 645, 2150 and 6450 mg/m^3) of styrene for 6h/day for 5 consecutive days. Animals were observed daily and body weights recorded before and during the exposure period. The animals were then sacrificed either immediately on the last day of exposure or after a 24-hr recovery period.

Styrene did not have marked toxic effects at the exposure levels tested; however, the animals in the high-exposure group showed signs of irritation (salivation) and of CNS depression (reduced attention) at the beginning of the exposure period. A statistically significant 2-fold increase in serum LH level was noted in the mid- and high- dose group of animals sacrificed immediately after exposure with consequential 2-fold increase in testosterone concentrations in these groups compared to controls. However, the effects were completely resolved within 24h because the observation was not seen in the respective recovery groups sacrificed 24h after. The concentrations of GABA and GLU in the mediobasal hypothalamus and GLU concentration in the striatum were unaffected by styrene exposure although a significant increase (by 47% and 41%) in striatal GABA concentration was observed in the recovery group animals exposed to 500 and 1500 ppm of styrene but not in the 500 and 1500 ppm animals sacrificed immediately after the end of exposure. Although the biological plausibility of this latter finding cannot be entirely dismissed, the toxicological significance of an increase in GABA levels in the striatum but not in the hypothalamus with no effects on levels of GLU in either the hypothalamus or the striatum 24h after the end of exposure is rather doubtful.

In summary, a transient increase in serum LH and T levels was seen in male rats exposed for 5 days to 500 and 1500 ppm (≈ 300 and 900 mg/kg/day) styrene. However, no related changes were observed in the concentrations of GABA and GLU in the hypothalamus or striatum. It is noted that, in contrast to this finding, a decrease in plasma T levels was observed in mice given a low oral dose of 12 mg/kg/day styrene in drinking water for 4 weeks (Takao *et al*., 2000; see mice section). Overall, in view of inconsistent results between studies, this isolated finding cannot be considered suffcient evidence of styrene-induced reproductive impairment.

Studies in rats investigating specific organ toxicity: kidney

A study specifically designed to investigate the nephrotoxic potential of styrene exposure in Sprague Dawley rats is available (Mutti *et al,* 1999). A group of 10 female rats was exposed whole-body to styrene vapour (99/9% purity) at 300 ppm, for 6 hours/day, 5 days/week for 12 weeks. A second group of animals was administered 2mg/kg of the known nephrotoxicant adriamycin (ADR) by intravenous injection on day 1 and 15 of the study, with no exposure to styrene. A third group of animals received both the styrene and ADR treatments. Finally a

fourth group of animals served as controls. Body weights and any clinical signs of toxicity were noted throughout the study. The urinary excretion of total protein and individual proteins (albumin, fibronectin, retinol-binding protein and 16Kd Clara cell protein) was measured for each group of animals monthly. At the end of the 12-week exposure period extensive kidney histopathology (including examinations for glomerular sclerosis, interstitial fibrosis, interstitial cellular infiltrate hyalinosis and the distribution and density of proliferating cells) was conducted and any tissue alterations graded according to the arbitary scale. Only the consequences of styrene exposure alone are reported here.

No deaths were reported in styrene-exposed animals and no reports of body weight changes or clinical observations were presented. In styrene-exposed animals some urinary indicators of kidney function were "slightly" but statistically significantly elevated, compared with untreated controls. Kidney weights in styrene-exposed animals were comparable with controls. At histopathological examination the scores for kidney interstitial fibrosis, cystic dilatations and hyaline were all statistically significantly elevated in styrene-exposed animals compared with controls. Scores for cellular infiltrates in styrene-exposed animals were comparable with controls. Overall, in itself the study suggests that repeated exposure of female SD rats to 300 ppm styrene for 12 weeks resulted in increases in the urinary excretion of proteins and histopathological changes in the kidney. However, the absence of similar findings in the rat in other repeated exposure studies of longer duration and at higher exposure concentrations indicates that these results are probably unreliable.

In a briefly-reported study, 10 male and 10 female Sprague Dawley rats were exposed to 0 or 135 ppm styrene, for 7 hours/day, 5 days/week for 13 weeks (Viau *et al,* 1987). Only the kidney (weight, function (glomerular filtration rate, urinary concentration ability) and histopathology) was investigated. No adverse effects on the kidney were observed in this study.

Studies in rats investigating specific organ toxicity: lungs

In an unpublished study specifically designed to investigate the effects of styrene exposure on the lungs, groups of male and female CD rats (5/sex/group) were exposed whole-body to styrene vapour (99.9% purity) at 0 or 500 ppm, for 6 hours/day, 5 days/week for up to 2 weeks (Green, 1999a). Groups of 5 animals were sacrificed at 17 hours after the 1st, 5th, 6th and 10th exposure, and the lungs removed for histopathological examination and quantitation of cell division rates (3 days prior to sacrifice each animal was fitted with a BrdU mini-pump). No treatment-related macroscopic or microscopic findings were observed in the lungs of any of the exposed animals. No increases in the labelling indices or in cell division were observed in any region of the lungs of treated animals at any time point.

A mechanistic study investigating the in vivo lung effects of styrene in rats and mice exposed by whole body inhalation is available (Gamer et al., 2004). Only the rat findings are reported here; the mouse findings are described under the "mice" heading of this section. Female CD rats (10/group) were exposed to 0, 688 or 2150 mg/m^3 (0, 160, 500 ppm) styrene for 6h/day for 1 or 5 exposures and sacrificed immediately at the end of the exposure period. Lung lavage was performed on 5 animals per group for each exposure duration. Cytological and humoral analyses were conducted together with the measurement of levels of proteins and CC16 (Clara cell specific protein), lactate dehyrogenase, alkaline phosphatase, γ-glutamyltransferase, N-acetyl-β-D-glucosaminidase and catalase activities. The blood of the remaining animals was sampled and the lungs, together with the trachea and larynx were removed for histopathological examination.

Statistically significant reduction in body weights was observed in the 160 and 500 ppm group. The CC16 level and the measured enzyme activities were unaffected by styrene exposure. No exposure-related histopathological findings were seen in the respiratory tract

and lungs of any of the exposed animals at the end of the exposure periods. Overall, this study showed that inhalation exposure up to 500 ppm styrene did not induce any Clara cell toxicity in rats.

Studies in rats investigating specific organ toxicity: nervous system

In a study of neurotoxicity parameters only, groups of 8 male Wister-derived rats were exposed to 0, 350, 700 or 1400 ppm styrene for 16 hours/day, 5 days/week for 18 weeks (Kulig, 1989). There was a recovery period of 6 weeks. Automated assessment of coordinated hindlimb movement and tests for grip strength, spontaneous activity, peripheral nerve conduction time and visual discrimination performance learning test were carried out at 3-week intervals during exposure and the recovery period (animals were trained on the apparatus for each test prior to exposure). Hearing was not investigated. No significant effects on coordinated hindlimb movement were detected in any exposure groups. A small decrease in forelimb grip strength was observed in animals exposed to 700 and 1400 ppm, from week 9, but the difference was not statistically significant. When the results from individual test days were analysed, a decrease in hindlimb grip strength of statistically significance was apparent in animals exposed to 700 and 1400 ppm at weeks 12 and 15; however no significance was attained when the date were analysed across exposure days and no such effects were noted any animals at week 18, nor in the post-exposure recovery period (at weeks 21 or 24). A decrease in response time in the visual discrimination performance learning test was noted in week 1 only, particularly at the highest concentration on day 1. Overall, the results of this study suggest that at exposure concentrations of up to 1400 ppm for 18 weeks, styrene did not express significant neurotoxicity; the delayed visual learning observed in animals exposed to 1400 ppm for the first week of exposure only (at later times, the 1400 ppm group was comparable with controls) may be indicative of a transient reversible CNS depressive effect.

A 13-week rat study was conducted in order to assess the neurotoxic potential of styrene (Albee *et al,* 1992). Groups of 14 male Fischer 344 rats were exposed to 0, 50, 200 or 800 ppm styrene for 6 hours/day, 5 days/week. Animals were observed daily and bodyweight measured weekly. A functional observational battery (including observations of salivation, abnormal movements or behaviour) and hind limb grip strength performance test were conducted monthly. Evoked potential tests (12 rats per group) were included after 13 weeks. Neuropathology at terminal necropsy was performed on 8 rats per group after electrophysiological tests were carried out. There were no exposure-related effects on mortality, bodyweight, functional observations, or grip strength and no treatment-related alterations in histopathological lesions in nerve tissues or limb muscles, other than in the auditory system (see later section).

Yamamoto *et al* (1997) studied the effects of styrene on nerve conduction velocity in rats; this study is also reported in Teramoto *et al* (1993). Groups of 8 Wistar rats aged either 4 or 8 weeks old at the start of the experiment were exposed whole-body to airborne concentrations of 0, 200 or 2000 ppm styrene for 8 hours/day, 5 days/week for 32 weeks. Nerve-conduction velocity of the tail nerve (using three sampling points between the base and end of tail) was assessed every two weeks from age 8 weeks (i.e. from the start of the experiment for older rats and after 4 weeks of exposure for young rats) until 16 weeks after the end of exposure. The following measurements were made at each of these times: motor nerve conduction velocity (MCV), distal latency (DL) and sensory nerve conduction velocity (SCV); the latter was determined for distal, proximal and whole tail areas. Body weight was slightly reduced (~5%) at 200 ppm compared to controls from week 18 in younger rats (but not older) and recovered after the end of exposure. Body weight was reduced by 15-30% in both ages of animals at 2000 ppm. No differences between treated and controls groups were observed in either MCV or DL at any time. Slight differences in distal, proximal and whole-

body SCV between the 200 ppm group and controls were occasionally observed (i.e. in one or two weeks out of the study) but these were not consistent and in some cases the values were slightly lower and in others, slightly higher. In contrast, although not seen at all weeks, in general the SCV of all three types were lower at 2000 ppm compared to controls over most of the exposure period, and in some cases after, in both ages of animals. The data were presented graphically but it would appear that the reductions in SCV seen at 2000 ppm were by around 5-10% compared to controls. Overall, in this study no effects were seen on tail nerve conduction velocity after exposure of rats to 200 ppm styrene with slight but consistent reductions in SCV at 2000 ppm.

In a similar study, exposure of rats to 300 ppm styrene for 6 hours/day, 5 days/week for 11 weeks resulted in an increase in tail motor nerve conduction velocity after 6 weeks, but no difference between control and exposed groups at 8 and 11 weeks (Seppalainen *et al,* 1978). This result is in contrast to the findings of the study summarised above. No other parameters were measured and the experiment was only reported in brief.

The effects of styrene on operant behaviour in rats has been investigated (Fumiko,1988). Groups of 6-7 (13 for controls) male JCL:SD rats were exposed whole-body to 0, 50, 150, 500 or 1000 ppm for 4 hours/day, 5 days/week for 3 weeks. For the study of Fixed Ratio Food Reinforcement schedule 30 (FR30) this exposure pattern was repeated three times with two week intervals between exposures. For the Low Response Ratio Differential Reinforcement Schedule 20 seconds (DRL20) only a single three week exposure pattern was employed. In the FR30, rats were trained prior to exposure to press a lever 30 times in order to obtain a food pellet. After the end of each four hour exposure period, FR30 was measured for a 20 minute period and the number of lever presses and amount of food acquired recorded. In the DRL20 rats were trained to obtain food by pressing a lever but with at least 20 seconds between each operation. The number of times the lever was pressed, the number of food pellets delivered and the ratio of leverpress/pellets delivered was recorded.

Exposure to styrene had no effect on body weight. FR30 was essentially unaffected compared to control at 50 or 150 ppm (a small non-statistically significant reduction in the number of lever presses was observed). The number of lever presses was reduced compared to controls at 500 (by 20%) and 1000 ppm (by ~35-50%). However, there was recovery during each two-week exposure-free period and after the end of the exposures, suggesting that the effects may reflect an acute response immediately following exposure. For DRL20, data were only presented for 50 and 1000 ppm. No difference in the number of lever presses, food acquired for the ratio of the two was observed between the 50 ppm and control groups. At 1000 ppm the number of lever presses was increased and good acquisition and ratio was reduced compared to controls. These differences were observed during the first half week and the end of the exposure period but thereafter showed a trend to recovery and from the second week returned to pre-exposure levels. This study demonstrated that exposure to styrene at 500 ppm and above affected the operant behaviour of rats but no significant effects were seen at 50 or 150 ppm.

Several studies have explored the effects of styrene inhalation on brain biochemistry in rats. In one, groups of 8 male rats inhaled a control atmosphere or styrene for 12 hours/day (Mutti *et al,* 1984). The control group was exposed for 7 days and styrene-exposed groups inhaled 750 or 1500 ppm for 3 days or 1500 ppm for 7 days. Additional animals (20/group) were included for the turnover experiment. Animals were sacrificed 8 hours after the end of the exposure period. Brains were removed immediately and tissue from the hypothalamus, median eminence (tubero-infundibular), striatum, cerebellum, hippocampus, and cortex areas was dissected and frozen. Catecholamine and homovanillic acid levels were assessed. Dopamine and homovanillic acid were measured in the striatum and tubero-infundibular areas and norepinephrine levels were reported for all regions sampled. In

samples from the groups exposed for 7 days, the turnover of dopamine are norepinephrine was determined by measuring catecholamine levels after intraperitoneal administration of α-methyl-p-tyrosine, which inhibits the synthesis of catechols via inhibition of tyrosine hydroxylase activity. Relative to controls, there was a marked dose-dependent decrease in dopamine in the striatum of animals exposed to 750 and 1500 ppm styrene and in tubero-infundibular areas at 1500ppm, accompanied by an increase in homovanillic acid in the same regions. Norepinephrine levels were not altered. The dopamine turnover rates were similar in control and exposed groups, suggesting that no increase in the activity of monoamine oxidase (MAO) could be detected in this study.

The effects of styrene inhalation on brain MAO-A and B activities have been investigated in another study (Coccini *et al*, 1999). Groups of 7-8 male Sprague Dawley rats were exposed whole-body to atmospheric concentrations of either 50 ppm styrene for 6 hours/day, 5 days/week for 13 weeks or 300 ppm for 6 hours/day, 5 days/week for 4 weeks; controls were sham-exposed under similar conditions. Further groups of 4 or 5 rats received daily i.p. injections of 100 or 400mg/kg/day styrene for 14 days, controls receiving corn oil. The rats were killed immediately after the last exposure, the brain dissected into the cerebral cortex, cerebellum striatum, hippocampus, brainstem and remaining brain. MAO-A and B activities were then determined in these brain regions. Comparative studies on MAO activities *in vitro* using untreated brain tissues exposed to styrene, styrene oxide, madelic acid, phenylglyoxylic acid and styrene glycol were also performed.

Inhalation exposure to both 50 or 300 ppm was stated to induce signs at lethargy during exposure but no changes in body weight, brain weight or brain protein content were observed. MAO-A activity was unaffected by treatment with styrene. MAO-B activity was reduced across all brain regions by exposure to both 50 ppm (38-50% across all regions studied) and 300 ppm (20-34% reductions across the regions). Following i.p. dosing, MAO-A activity was unchanged and MAO-B activity was only reduced statistically significantly in the brainstem (by about 45% for both doses). No effects were seen on either MAO-A or B activities in the studies performed *in vitro.* Overall, changes were seen in MAO-B activities in this study but their toxicological significance is unknown.

The effect of styrene, with and without alcohol, on brain and hepatic non-protein sulfhydryls (NPS), lipid peroxidation and enzymes has been investigated in rats (Coccini *et al*, 1996). Groups of five male Sprague-Dawley rats received either a liquid alcohol (5% w/v; 36% daily caloric intake) or isocaloric liquid (alcohol replaced buy dextrine/maltose) diet for three weeks. During the second and third weeks groups on each diet were exposed whole-body to either 0 or approximately 325 ppm of styrene for 6 hours/day, 5 days/week. Animals were killed immediately after the last exposure, the brains and liver removed and the following assessed in each tissue: NPS (as a measure of GHS), lipid peroxidation, protein content, hepatic aniline hydoxylase and aminopurine *N*-demethylase and the content of cytochrome P450 and Cyt b_5. Styrene blood levels were also measured.

Rats exposed to styrene, or alcohol or both combined had lower body weights than controls (by 15-20%) at the end of exposure. No changes were seen in brain or hepatic lipid peroxidation or protein content. NPS levels were reduced in the brain (by 30%) and liver (by 50%) following exposure of rats to styrene on isocaloric diet and on alcohol diet (brain by 60%, liver 50%). Styrene alone was found to increase hepatic enzyme activities and the content of cytochrome P450 and Cyt b_5.

Another inhalation study was designed to assess potential effects on behaviour and changes to the brain following exposure of rats to styrene (Savolainen *et al,* 1980). Groups of 26 male Wistar rats were exposed to 0 or 300 ppm styrene for 6 hours/day, 5 days/week for 4-17 weeks. Six rats from each group were withdrawn from exposure after 8 weeks and sacrificed in groups of 3 after one or two weeks free of exposure. The remaining rats were killed in groups of 5 after 4, 8, 13 and 17 weeks of exposure. Cerebral samples taken were used to

isolate glial cells. The glial glutathione (GSH) concentration and activity of acid proteinase and NADPH-diaphorase were determined. Brain samples were taken from the animals that had been withdrawn from exposure at 8 weeks and RNA content and activity of acid proteinase and NADPH-diaphorase analysed. Behaviour was analysed in an open field situation after 4, 9 and 13 weeks of exposure. Body weight was unchanged relative to the control group. Generally the analyses indicated no consistent pattern of differences in styrene-exposed animals when compared with the control group. There were also no significant changes in behavioural parameters in styrene-exposed animals.

In an earlier study by the same group, the effects of styrene on brain proteins and brain and serum enzyme activities were investigated in rats exposed by inhalation (Savolainen and Pfaffli, 1977). Groups of 40 adult male Wistar rats were exposed to either 0 or 300 ppm styrene by whole body-exposure for 6 hours/day, 5 days/week for between 2 and 11 weeks. Five rats from each of the exposed and control groups were killed at 2 weeks and then at weekly intervals from weeks 4-11 from the beginning of exposure. Brain, spinal cord, blood and perirenal fat were taken. The right hemisphere of the brain and perirenal fat were assessed for the styrene content. The left hemisphere of the brain was assessed for protein and RNA content and for acid proteinase (AP), acetyl cholinesterase (ACh) and creatine kinase (CK) activities. A water-soluble brain fraction and spinal cord axonal fraction were analysed for protein profile by polyacrylamide gel electrophoresis (PAGEP). Serum non-specific cholinesterase and CK activities were also measured.

Animals were stated to be “somnolent” during exposure in the early stages of the study with this effect “levelling out” (it is unclear whether this meant signs were less prominent or were seen to be at the same degree) towards the end of the experiment. No other clinical signs of altered neurological function were observed. Styrene levels in brain and perirenal fat increased during the first four weeks of exposure but subsequently dropped to between one-half (fat) and one-fifth (brain) of these levels by the 11th week of exposure. Occasional small (5-10%) statistically significant differences between exposed and control groups were seen in brain protein and RNA content, and AP, ACh and CK activities. These were seen generally towards the end of the exposure period when brain styrene levels were lower, were inconsistent (i.e. would be different from concurrent controls at one time point but not the next) and generally fell within the range of control values seen throughout the whole experiment. Serum non-specific cholinesterase activity was lower by 25-30% after 2-4 weeks of exposure to styrene but was subsequently similar in both exposed and control groups. No changes in protein profile were observed in PAGE analysis of the water-soluble brain fraction. The expression of one protein near the cathode in the axonal spinal cord preparation was reduced from week 9 of exposure but major protein bands remained unchanged. Overall, no consistent effects were seen in the brain biochemistry endpoints measured in this study in rats exposed to 300 ppm styrene for up to 11 weeks. An early reduction in serum non-specific cholinesterase activity was seen but subsequent adaptation appears to have accommodated for this reduction.

Rosengren and Haglid, (1989) studied brain protein in rats exposed continuously to styrene for 90 days. Groups of 8 (exposed) or 16 (control) Sprague Dawley rats inhaled 0, 90, 320 ppm. Animals were then kept for 4 months free of exposure, sacrificed, the brains removed, and various regions of the brain isolated by dissection. Tissue samples were homogenised and the astrological markers S-100 and glial fibrillary acidic proteins (GFAP) measured. Body and brain weights and S-100 were not significantly different between control and exposed groups. Relative to the controls, GFAP concentrations in the 320 ppm group were significantly increased in the sensory motor cortex and in the hippocampus. The appropriate toxicological interpretation of this finding is unclear.

Studies in rats investigating specific organ toxicity: auditory systems

A 13-week rat inhalation study explored the ototoxic potential of styrene (Albee *et al,* 1992). Groups of 14 male Fischer 344 rats were exposed to 0, 50, 200 or 800 ppm styrene for 6 hours/day, 5 days/week. Animals were observed daily and bodyweight measured weekly. Evoked potential tests (12 rats per group) were included after 13 weeks. Cochleas were removed from 4 rats in the control and 200 ppm groups and 3 rats from the 800 ppm group and sections specially processed for pathology. There were no exposure-related effects on mortality or bodyweight. Histopathological investigations revealed lesions in the organ of Corti of animals exposed to 800 ppm styrene (two tissue samples were examined). These lesions were characterised as the loss of two outer hair cells per cross section from the upper basal turn, and the occasional absence of an outer hair cell from the lower middle turn. There were no alterations involving the inner hair cells, the Deiters' cells or the pilar cells. The organ of Corti was not affected in animals exposed to 0 or 200 ppm styrene (no tissues were examined at 50 ppm). There were no treatment-related alterations in somatosensory evoked potential from the sensory cortex or the cerebellum. However in the 800 ppm group, auditory brainstem response (ABR) thresholds were elevated by approximately 40 dB at 16, 25 and 30 kHz. Hair cell loss at 800 ppm occurred in the cochlea in areas that relate to mid – high frequency (15-30 kHz) hearing. In this study, the NOAEL for ototoxicity was 200 ppm, with evidence of damage and impairment in the auditory system at the next highest dose of 800 ppm.

A further investigation of ototoxicity involved groups of 12 male Fischer 344 rats exposed to 0 or 800 ppm styrene for 14 hours/day, 5 days/week for 3 weeks (Yano *et al,* 1992). In styrene-exposed animals brainstem auditory evoked potentials were "slightly" affected at 4 kHz and moderately-severely altered at 8, 16, 30 kHz. Following testing and sacrifice, cochleas were removed from 4 controls and 4 of the styrene-exposed animals with the largest changes in auditory evoked potentials. In the styrene-treated rats, outer hair cell loss was observed in the organ of Corti which corresponded with the hearing effects at mid frequencies. Where damage to the cochlea was severe, occasionally inner hair cells (IHC) were missing. In another study, brainstem auditory evoked response thresholds were elevated at all frequencies tested, [4, 8 and 16 kHz], in male Fischer 344 rats exposed to styrene at 800 ppm or above for 14 hours/day daily for 3 weeks (Pryor *et al,* 1987). The response was greater at 8 and 16 kHz (humans can hear in this range). Neither study explored the effects of styrene exposure at concentrations lower than 800 ppm.

Rebert *et al* (1993) exposed groups of 6 rats to 0, 500, 1000, 1500, 2000 or 2500 ppm styrene as part of an experiment to determine the effect of co-exposure to styrene and trichloroethylene on the auditory system. Animals were exposed for 8 hours/day for 5 days. The amplitude of the brainstem auditory evoked response (BAER) at 16 kHz was measured on the 10^{th} day after the end of exposure at all concentrations and latency was determined at 1000 ppm only. In the animals exposed to styrene only, there was no change in amplitude at 500 ppm but significant decreases in amplitude occurred at 1000 ppm and above when compared with the control group. Latency was significantly decreased at 1000 ppm.

The ototoxic potential of styrene in rats following inhalation exposure either alone or in combination with noise has been studied in detail by Makitie (1997). In a series of experiments, groups (10-12/group) of male Wistar rats were exposed whole-body to styrene alone, noise alone pr the two agents combined. Exposure conditions for styrene were 100, 300 or 600 ppm for 12 hours/day, 5 days/week for 4 weeks. Noise exposure conditions were broad-band non impulse noise in the frequency range 31.5-10 kHz with a sound pressure of 100-105 dB for 12 hours/day, 5 days/week for 4 weeks. Combined exposures were to the same noise conditions together with each of the three styrene exposure conditions. Controls were exposed to filtered air and normal noise levels. Because of the design of the study in

some cases (controls, noise alone and 600 ppm styrene alone) more than one group was included overall.

Ototoxicity was assessed electrophysiologically by auditory brainstem response (ABR) and morphologically using light and electron microscopy (LM and EM) ABR was assessed using cutaneously applied electrodes, with testing being carried out prior to exposure, immediately after exposure and then at specified intervals after exposure (generally about 3-6 weeks later). Auditory stimulus for ABR consisted of clicks with alternating polarity and tone bursts of 4 ms duration at 16 deliveries/second. The response to stimulus was assessed at frequencies of 1, 2, 4 and 8 kHz with testing being carried out on the right ear. ABR thresholds were defined as the lowest level giving reproducible responses in the system. Hearing loss for each rat was assessed by comparison of pre and post exposure thresholds as well as comparison against controls ABR latencies (as a measure of cochlear nerve conduction) were also measured in some rats and was defined as the time between stimulus onset to the peak occurrence of the first and fifth waves of the ABR. After the electrophysiological studies had been completed a number of morphological studies were carried out with both LM and EM. Both set of analyses were essentially designed to investigate the morphology of the organ of Corti, in particular effects of treatment on the inner and outer half cells (IHC and OHC).

No general signs of toxicity were observed during or after treatment. Body weight gain was reduced at all styrene exposure concentrations compared to controls when administered alone but was increased in noise exposed animals. No effect was seen on ABR latencies with any treatment. Styrene alone at 100 and 300 ppm had no effect on BAR thresholds compared to either pre-exposure or controls, did not increase loss of IHC or OHC and did not induce any increases in subcellular pathology examined by EM. Exposure to styrene at 600 ppm produced mild increases in both temporary and permanent threshold shifts (TTS and PTS) of about 1-3 dB compared to pre-exposure and control values. This electrophysiological difference was accompanied by severe OHC loss in the third row of cells with less loss seen in the second and first rows respectively: no loss in IHC was seen. EM studies showed subcellular changes in OHC including increased vacuolation in the cytoplasm, formation of vesicles and alteration of mitochondria (disruption of cristae and formation of membrane-bound spherical bodies). Noise alone caused a mean threshold shift ranging from 1.8 dB (at 8 kHz) to 9.3 dB (at 2 kHz), with effects most marked at the lower frequencies (1-4 kHz). Morphological analysis by LM did not reveal any increased loss of OHC or IHC compared to controls, although EM studies revealed some stereocilia irregularity and apparent loss of stiffness in hair cells. The combination of noise with 100 or 300 ppm styrene caused a flat increase in PTS of around 5-10 dB across the whole frequency range studied but this increase was about the same as for noise alone. No increase in IHC or PHC loss was seen under these combined conditions of exposure. The combination of noise with 600 ppm styrene, however, produced a much greater increase in PTS, which was greater than the simple summation of the response to noise and 600 ppm styrene alone, indicating a potentiation of effect. This electrophysiological change was accompanied by a loss of OHC, which was more severe than that seen with 600 ppm alone and there was also some loss if IHC. EM studies revealed structural changes such as increased vesiculation and vacuolisation of OHC cytoplasm and many cells looked to be in stages of degeneration. Overall, this study demonstrated that in rats ototoxicity assessed by electrophysiological and morphological techniques was induced by exposure to 600 ppm styrene but not at 300 ppm or less, indicating a threshold in this species somewhere between 300 and 600 ppm. The combination of noise and styrene exposure was found to potentiate the effect of styrene alone but only at a level where both agents were found to be ototoxic. Where styrene was administered at non-ototoxic concentrations with noise the severity of effects seen was only equivalent to those produced by noise alone.

In a further study, groups of eight male Long-Evans rats were exposed whole body to 0, 500, 650, 850, 1000 and 1500 ppm styrene for 6 hours/day, five days/week for four week (Loquet

et al, 1999). Prior to exposure rats were implanted with brain electrodes in order to record auditory stimulus evoked potentials. Audiometric testing (noise intensity thresholds for evoked potential across a range of frequencies from 2-32 kHz) was performed one month after implant at two days prior to styrene exposure and then at six weeks after the exposure period. Permanent threshold shifts (PTS) were calculated as the difference in sound intensity required to induce an evoked response between the pre and post exposure measured thresholds. Following audiometry testing histopathological and scanning electron microscopic (SEM) examination was used to assess damage to the inner and outer hair cells (IHC and OHC) of the organ of Corti. Counts of surviving hair cells were used to quantitatively assess the damage; no attempt was made to judge the condition of surviving hair cells.

PTS was elevated at exposure levels of 850 ppm and above. At 850 ppm there was a large PTS over the frequency range 16-20 kHz but no hearing loss at lower or higher exposure concentrations. At higher exposure levels hearing loss was measured across the frequency range with the effect increasing with airborne exposure concentration. Slight effects were seen at 650 ppm but no change in PTS compared to controls was seen at 500 ppm. Histopathology and SEM revealed no effects on IHC in treated or control animals and a background loss of OHC in controls. In contrast animals treated with 650 ppm and above styrene showed loss of OHC in all three rows with most loss seen at positions which are believed to correspond to the 4 and 20 kHz detection frequencies. The effects were most marked across the exposure range in the third row of OHC with progressively less damage in the second and first rows. This study demonstrates that styrene is ototoxic in rats following inhalation exposure at concentrations of 650 ppm and above, with a clear NOAEL being identified at 500 ppm. The effects measured by audiometry were accompanied by damage to the OHC in the affected groups.

In a subsequent report, the same group of workers using essentially the same techniques investigated the interaction between styrene and noise exposure in rats (Lataye *et al,* 2000). Groups of 16 male Long-Evans rats received implants to measure auditory stimulus evoked responses and were exposed to: noise alone (97 dB) octave noise centred at 8 kHz which was chosen where auditory sensitivity was expected to be highest) for 6 hours/day, 5 days/week for 4 weeks; styrene alone, whole-body at 750 ppm for 6 hours/day, 5 days/week for 4 weeks; styrene plus noise at these levels; air and normal noise levels. Audiometry was performed prior to exposure, on the day following the end of exposure and after a six week recovery period. Histopathology and SEM studies were performed as before, immediately after the audiometry studies.

Noise alone was found to increase evoked potential thresholds (i.e. affect hearing) at both time points after exposure over the frequency range 8-20 kHz, with peak effect seen at 12 kHz: some recovery was seen after the six week recovery period although shifts in evoked potential thresholds were still observed. Styrene alone was found to affect hearing over the range 16-20 kHz but the effects were not as great as noise alone, although subsequent recovery was not observed. When styrene was combined with noise, the induced hearing loss was observed over the frequency range 8-20 kHz (i.e. the same range as noise alone) but was found to be greater than the arithmetical sum for the two agents alone over the frequency range 6-12 kHz, indicating an interaction between the two agents.

Histopathological and SEM analysis showed a loss of OHC (about 17% loss) in the first row at a position associated with 17 kHz hearing frequency; OHC rows 2 and 3 showed slight losses in cells. Styrene induced large losses of OHC in row 3 at 20 kHz (86% loss) and 4 kHz (70% loss). OHC2 and OHC1 were less damaged than OHC3. Exposure to styrene and noise together induced a loss of hair cells in a pattern similar to that for styrene alone but of a more severe nature, with loss of 94% of cells in OHC3 at the 20 kHz position and 86% of cells in OHC3 at the 4 kHz position. Overall, this study demonstrated that when rats were exposed to the two agents, noise and styrene, each at ototoxic levels, the effects on auditory

function and histopathological damage were more marked than might be expected if the effects of the two were simply added, suggesting a positive interaction.

The ototoxic potential of styrene in rats and guinea pigs following inhalation exposure has been studied by Lataye *et al.* (2003). The guinea pig investigations of this study are reported under "Other species" and only the rat investigation is summarised here.

Groups of 5-6 male Long-Evans rats were exposed, whole-body, to 0 or 1000 ppm (4330 mg/m^3) styrene vapour, 6 hours/day for 5 days. At regular intervals during the exposure period urine samples were taken for measurement of urinary metabolites of styrene. Cochlear function was tested before, 20 minutes after the end of the 5 days of exposure, and 2 and 4 weeks post-exposure using cubic distortion product otoacoustic emissions (DPOAE) recorded from the external ear canal in anaesthetized animals. A satellite group of animals was anaesthetized immediately after the last styrene exposure, killed and subjected to blood sampling for analysis of styrene levels. An additional group of 5-6 male Long-Evans rats exposed, whole-body, to 1000 ppm styrene vapour, 6 hours/day for 5 days was sacrificed four weeks after the last exposure for removal and analysis of the aural bones and tissues.

The bodyweights of animals were recorded weekly – no differences from controls were noted during the course of this study. In styrene-exposed rats, DPOAE amplitudes were statistically-significantly decreased at 2 and 4 weeks post-exposure, although the magnitude of the effect was no greater at 4 weeks than at 2. Histologically, hair cell loss was observed. Blood styrene levels on completion of 5 days of styrene exposure were 22.8 μg/g. Urinary hippuric acid levels at 3 and 4 days post-exposure were around 4 g/g creatinine.

Overall, this study shows hearing loss in rats exposed to 1000 ppm (4330 mg/m^3) styrene for 5 days. Unfortunately, this study did not employ a range of different exposure levels to explore the dose-response relationship.

As part of a study investigating the combined effects of styrene and ethanol, groups of 10-11 male Long-Evans rats were exposed, whole-body, to 0 or 750 ppm styrene vapour 6 hours/day, 5 days/week for 4 weeks (Loquet *et al*, 2000). Audiometric thresholds for each animal were determined from 2 to 32 kHz using brainstem auditory evoked potentials before the start of the study and 6 weeks post-exposure. Urine samples were collected every day for the first week of styrene exposure and then on the 4^{th} day of each week for the remaining 3 weeks. Samples were analysed for mandelic acid (MA), phenylglyoxylic acid (PGA), hippuric acid (HA), and also for N-acetyl-β-D-glucosaminidase (NAG), γ-glutamyl-transpeptidase, total urinary protein, and urea. At termination, blood samples were taken for analysis of ALT and AST and glutamate dehydrogenase, and liver samples for analysis of CYP2E1. Histology examinations included counting hair cells in the organ of Corti from 5 animals and focussed only on auditory tissues.

The audiometry examination showed evidence of hearing loss at high and low frequencies (increased, permanent shifts in auditory threshold at 2, 16 and 20kHz). The cytocochleogram showed outer hair cell loss of approximately 86% in regions corresponding to 8 and 22kHz. There were no significant differences between styrene-exposed animals and controls in the urinary biomarkers of liver or kidney toxicity. Styrene induced a significant increase in the activity of CYP2E1 compared to controls. Overall, this study demonstrates hearing loss accompanied by histological damage in rats exposed repeatedly to 750 ppm styrene for 4 weeks.

In a time course experiment, male Long-Evans rats were exposed to 0 (16 animals) or to 1000 ppm styrene for 6h/day, 5 days/week, for either 1 (16 animals), 2 (12 animals), 3 (12 animals) or 4 (12 animals) consecutive weeks (Campo et al., 2001). Following exposure, groups of 4-8 animals were sacrificed for the histological analysis of the cochlea. Audiometric thresholds were determined in 8 animals per group prior to exposure, the day after exposure and after a 6-week recovery period. Surprisingly, hearing loss was similar

despite the differences in duration of exposure, but worsened after the end of the exposure. For example, the 16 kHz threshold shift was 20 dB at the end of the exposure period (for the 1-week exposure test) but increased up to 35 dB by 6 weeks post-exposure. The histological damage in the cochlea (large losses in the third row of the outer hair cells -OCH3-, less severe damage in the OCH2 and no lesions in OCH1 and IHC) was also similar for the different durations of exposure. Analysis by electron microscopy showed damage and disorganisation of the plasmalemma membrane. Overall, this study shows that ototoxicity (hearing loss accompanied by histological damage) in rats exposed repeatedly to 1000 ppm styrene does not worsen with duration of exposure (from 1 to 4 weeks). Hearing loss seems to progress after the end of the exposure period reaching its maximum at around 6 weeks post-exposure. This is likely to be the consequence of the damaged hair cells which are still dying off after the end of the exposure. This study also shows that styrene and/or its metabolites cause a serious disturbance of the membranous organisation.

Studies on other organic solvents such as toluene (Johnson and Nylen, 1995; Pryor et al., 1984) show that ototoxicity appears after relatively short exposures and that continued treatment does not enhance the intensity of the ototoxic response. A clear decrease in auditory sensitivity was seen in rats exposed to 1000 ppm toluene (22h/day, 7 days/week) for one month, but no hearing effects were found after 19 months of exposure with a shorter daily duration (6h/day, 5 days/week) (Nylen et al., 1987). Although no histopathology was conducted in this study, these findings show that the daily exposure concentration is the important factor in determining hearing loss and not the total length of exposure.

Styrene-induced hearing loss did not appear to be counterbalanced by central compensation (GABAergic adjustment in the inferior colliculs) in Long-Evans rats exposed to 700 ppm styrene 6h/day, 5 days/week for 4 weeks, but hearing loss caused by noise (97 dB SPL octave band noise centered at 8 kHz) did appear to be centrally compensated (Pouyatos et al., 2004).

Styrene-induced hearing loss was greater in 3-month old Long-Evans rats compared to 4-month old rats exposed to 700 ppm styrene 6h/day, 5 days/week for 4 weeks, but was not body weight-sensitive. These data suggest that younger adult rats may be more susceptible to styrene ototoxicity compared to older adult animals (Lataye et al., 2004).

Lataye et al (2005) compared styrene-induced ototoxicity in active rats and sedentary/ordinary rats and investigated the combined effects of noise and styrene on hearing. Groups of eight male Long-Evans rats were exposed whole body under sedentary/ordinary conditions to 0, 500, 650, 850 or 1000 ppm styrene for 6 hours/day, five days/week for four week. Additional groups of 4 male Long-Evans rats, forced to run (for 2 minutes every 3 minutes) in a special wheel during the exposure, were exposed whole body to 0, 300, 400, 500 or 600 ppm styrene for the same exposure duration. Furthermore, groups of 6 active male Long-Evans rats were exposed to 0, 400 ppm styrene alone, noise alone (an octave band noise centered at 8 kHz) or styrene (400 ppm) and noise combined. Audiometric testing was performed prior to styrene exposure and at six weeks after the exposure period. Following audiometry testing histopathological and scanning electron microscopic (SEM) examination was used to assess damage to the inner and outer hair cells (IHC and OHC) of the organ of Corti.

In the sedentary animals, hearing loss and OHC cell damage were observed at 650 ppm and above, but not at 500 ppm. In the active rats, functional and histological damage was observed at 400 ppm and above, but not at 300 ppm. These results show that the ototoxic potency of styrene exposure depends on the physical activity of the animals as this is related to the ventilation rate and, in turn, to the uptake of the chemical via the lungs. Overall, based on these findings, NOAEC values of 500 and 300 ppm can be identified in sedentary/ordinary rats and active rats, respectively.

In the experiment investigating the combined effects of noise and styrene, noise alone or styrene alone were found without effect; however, both heraing loss and OHC cell damage were observed in the animals exposed to noise and styrene combined. These results suggest that styrene-induced ototoxicity can be potentiated by exposure to noise.

Campo et al. (1999) investigated whether styrene-induced ototoxicity is caused by “tissue intoxication” or by “fluid contamination”. Long-Evans rats were exposed to styrene in air at a concentration of 1750 ppm for 6 hours on the first day and a further 4 hours the following day. Immediately after exposure, blood, brain, auditory nerves, the organ of Corti, cerebrospinal fluid and inner ear fluid were sampled and the styrene concentration in each tissue determined by gas chromatography. Styrene was found in the organ of Corti, the nerves and the brain but not in the cerebrospinal and inner ear fluids. Based on these data the authors proposed that styrene-induced ototoxicity is caused by “tissue intoxication”. Styrene, transported by blood coming from the stria vascularis or the spiral prominence, diffuses through the outer sulcus to reach the lipid-rich Hensen’s cells. These cells are in close connection with the Deiters cells that are directly located under the outer hair cells (OHC). Thus the target cells are reached by diffusion of styrene. This explains why the hair cells are lost sequentially from OHC3 to OHC1 as diffusion continues.

Clear evidence of ototoxicity (both functional and histological) has been seen in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks’ duration. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tend to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. The underlying toxicological mechanism has not been clearly elucidated. This effect should be regarded as of potential relevance to human health. The histological damage consists of the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irriversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organisation of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. On this basis, it is considered that the NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively are a more accurate starting point for risk characterisation than the NOAEC of 200 ppm for 13 weeks.

Studies in rats investigating specific organ toxicity: Ocular system

Groups of 10 female Sprague-Dawley rats were exposed, whole-body, to 0 or 300 ppm styrene 6 hours/day, 5 days/week for 12 weeks (Vettori *et al*, 2000). Retina were removed post-mortem; the right retina was studied for tyroxine hydroxylase-immunoreactive (TH-IR) amacrine cell count, and the left retina was analysed for dopamine (DA), catecholamine (CA), tyrosine hydroxylase (TH), homovanillic acid (HVA), 3,4-dihydroxy-phenylacetic acid

(DOPAC), and glutathione levels. Amacrine cells are neurons that produce dopamine, which is assumed to modulate the retinal cone-horizontal cell transmission that is involved in colour vision.

The number of large amacrine cells was reduced by around 30% in styrene-exposed rats when compared to controls. The density of small amacrine cells was unaffected. Dopamine and DOPAC content were lower than controls (22% and 17% respectively), as were glutathione levels (28%). Overall, this study showsminor effects on the number of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks. However, it is considered that, in the absence of any associated conventional histopathology and given the lack of information on what are the 'normal' levels of dopamine and glutathione in the retinal cells of rats, the rather minor morphometric and biochemical changes observed are unlikely to represent a toxic response. It should also be noted that this was a non-standard test which did not involve the use of positive and negative control substances; hence, this questions the reliability of the reported findings which could just be an artefact of sample preparation/handling.

Summary of repeated inhalation exposure studies in rats

A variety of repeated inhalation exposure studies in rats have been conducted. Two well-characterised target sites have been identified: the nasal epithelium and the ear. In relation to nasal epithelium damage, a NOAEC has not been identified, with chronic inflammatory changes (mild in degree and confined to the olfactory epithelium with no effects on Bowman's glands or olfactory nerve fibres), having been seen with long-term daily exposure to 50 ppm styrene, the lowest concentration explored in a 2-year study. Nasal lesions have also been oberved after 13 weeks of exposure at 500 ppm and above and in the high exposure group (500 ppm) of the F_0 and F_1 generations (treated approximately for 10 weeks) in a recent 2-generation study (see section 4.1.2.9.2). It has been shown that the cytochrome P450-mediated metabolism of styrene to the reactive metabolite styrene oxide and to other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol) is a crucial factor in the expression of nasal epithelium toxicity. It is important to consider that cytochrome P450-catalysed metabolic activation of styrene to styrene oxide in human nasal epithelial tissue appears to be negligible (see toxicokinetics section). In relation to 4-VP and its downstream metabolites, studies with rat, mouse and human lung microsomes have shown that the relative formation rates of these chemical species indicate that the mouse ability to produce these metabolites is greater than in the rat (14-79% of the mouse concentrations) and even greater than in humans (1.5-5% of the mouse concentrations) (Bartels et al, unpublished, 2004). A similar toxification of styrene via 4-VP and its downstream metabolites may also be presumed to be mediated by CYP2F2 in the nasal tissue especially of mice. Hence, it can be concluded that the nasal epithelium damage induced by styrene in rats is not of relevance in relation to the potential repeated dose effects of styrene in humans at relevant levels of exposure.

In relation to effects on the ear, clear evidence of ototoxicity (both functional and histological) has been seen in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks' duration. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tend to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene.

The underlying toxicological mechanism has not been clearly elucidated. This effect should be therefore regarded as of potential relevance to human health. The histological damage consists of the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irriversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organisation of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. On this basis, it is considered that the NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively are a more accurate starting point for risk characterisation than the NOAEC of 200 ppm for 13 weeks.

There is no convincing evidence for repeated inhalation exposure to styrene producing damage to, or dysfunction of the central nervous system in rats. CNS depression (narcosis) has been observed, but this was a transient and reversible acute effect rather than the consequence of repeated exposure. Variable results have been obtained in studies investigating elements of brain biochemistry, but none of the differences detected between styrene-exposed and control animals are interpretable within an established framework of well-validated neurotoxicological criteria.

In one single non-standard investigation, rather minor effects on the number of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks have been reported. However, it is considered that, in the absence of any associated conventional histopathology and given the lack of information on what are the ‘normal’ levels of dopamine and glutathione in the retinal cells of rats, the rather minor morphometric and biochemical changes observed are unlikely to represent a toxic response.

Mice

General studies in mice investigating a range of toxicological endpoints

In a well conducted combined chronic toxicity/carcinogenicity study, groups of 70 male and 70 female CD-1 mice were exposed whole-body to 0, 20, 40, 80 or 160 ppm styrene monomer vapour (98.8 to 99.5% purity) for 6 hours/day, 5 days/week for up to 104 weeks (Cruzan *et al,* 1998). Ten animals per sex per group were sacrificed at weeks 52 and 79 for interim analyses. The remaining 50 animals/sex/group were maintained for the remainder of the study duration (see section 4.1.2.8.1 for study details). Two females at 160 ppm were found dead or moribund during week 2 of exposure. Deaths were considered to be treatment-related and as a consequence of the hepatocyte necrosis in these animals observed at necropsy. In these decedents metaplasia of the olfactory epithelium of the nasal passages was also observed. No other treatment-related deaths occurred. The only clinical sign of toxicity observed was increased activity in animals at 160 ppm, for the first week of exposure, compared to controls. Activity levels were comparable with controls throughout the remainder of the study.

Relative to controls, body weight gain was unchanged in males and females at 20 ppm. During the first 13 weeks of the study in both males and females at 40, 80 and 160 ppm, body weight gain was statistically significantly reduced compared with controls, by 11, 11 and 33% in males and by 12, 12 and 25% in females. A reduced body weight gain was

sustained in these animals throughout the remainder of the study, although statistical significance was only maintained in males at 80 and 160 ppm (body weight gain reduced by 23 and 31%) and in females at 160 ppm (body weight gain reduced by 15%). Food consumption in males at 80 ppm and in males and females at 160 ppm was statistically significantly reduced during the first 13 weeks of the study compared with controls (by 8 to 10%), and this reduction was maintained throughout the duration of the study. Water consumption was increased in a dose-responsive manner in animals at 40, 80 and 160 ppm by 7 to 23%, compared with controls, during the first 4 weeks of the study. Thereafter until week 79 of the study water consumption was slightly reduced in all exposed animals. Immediately prior to study termination water consumption was comparable in all animals. Haematological, clinical chemistry and urine analyses revealed no treatment-related changes, in any of the animals examined. Opthalmoscopic examination did not reveal any treatment-related changes in any of the exposed animals.

At weeks 53 and 79 for both sexes and weeks 98 for females and 104 for males, absolute and relative organ weights of treated animals were comparable with controls. No adverse macroscopic pathological findings were observed at week 53. At week 79 and 98 (females) or 104 (males) there was an increase in the number of lung masses in exposed animals, but this did not appear to be dose-related. Microscopic examination at weeks 53, 79 and 104 revealed treatment-related changes in the nasal passages of exposed animals. The changes were predominantly located in the dorso-median airway but extended to the dorso-lateral and medial median airways in the later examinations. Changes were observed in animals exposed to 40, 80 and 160 ppm styrene at week 53 and in all exposed animals at weeks 79 and 98/104. The observed changes consisted of respiratory metaplasia of the olfactory epithelium, dilatation of the Bowman's gland and respiratory metaplasia in the Bowman's gland, and atrophy of the olfactory nerve fibres. In animals at weeks 79 and 98/104 at 80 and 160 ppm (but not at 20 or 40 ppm), in which there was marked metaplasia and loss of nerve fibres, there was also a marked reduction in the depth of lamina propria (with loss of Bowman's glands) and a focal loss of bone from the underlying turbinates. Other treatment-related changes in these animals were focal degenerative lesions in the olfactory epithelium (necrosis/degeneration/atrophy), the presence of rosettes or prominent Bowman's glandular elements, presence of eosinophilic inclusions and of inflammatory debris in the dorso-median airway and an increased incidence of ductal ectasia with or without glandular proliferation. In males at 104 weeks there was also and increased incidence of epithelial hyperplasia in the nasolachrymal duct, compared with controls. The severity of all of these changes was dose-related, being minimal at 20 ppm, moderate at 40 ppm and marked at 80 and 160 ppm.

Treatment-related changes were also found in the lungs of exposed animals (at 40, 80 or 160 ppm at week 53, and in all groups including 20 ppm, at weeks 79 and 98/104). These comprised hyperplasia in the terminal bronchioles and focal bronchiolar-alveolar hyperplasia and fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct. No other treatment-related non-neoplastic findings were observed in exposed animals. Neoplastic changes are detailed in section 4.1.2.8.1. Overall, these data indicate that in the mouse the nasal passages and lungs are the target organs for styrene toxicity following exposure via the inhalation route under the conditions of this study. A NOAEC cannot be determined from this study, chronic inflammatory changes being observed at 20 ppm, the lowest exposure level tested.

Another study is available in which groups of 10 male and 10 female CD-1 mice were exposed whole-body to 0, 50, 100, 150 or 200 ppm styrene for 6 hours/day for 13 weeks (Cruzan *et al*, 1997). A satellite group of 30 males was exposed by the same regimen for 2, 5 or 13 weeks, at which time lung and liver cell proliferation was determined by BrdU labelling. Animals were assessed during the study for deaths, body weight changes and clinical signs of toxicity. Extensive analyses of haematological and clinical chemistry parameters were conducted. Extensive gross pathological and histopathological examination

was performed. Additional satellite groups of 5 mice/sex/group were examined at week 1 with respect to serum sorbitol dehydrogenase (SDH) and alanine transferase (ALT), bile and acid levels and liver histopathology.

Two female mice exposed to 200 ppm died during the first week of the study. Evidence of centrolobular necrosis and congestion was found in the livers of the decedents and was reported as the cause of death. Abnormal histopathology of the nasal passages (atrophy of the olfactory epithelium) was also observed in the decedents. In relation to the surviving animals, (females at 200 ppm were cold to touch, lethargic and had slow respiration during the first week of exposure. No other clinical signs of toxicity were observed. In males at 200 ppm body weights and food consumption were reduced (no quantitative data reported). Organ weights, haematological and clinical chemistry parameters were comparable with controls in all animals investigated at each time point.

Abnormalities in the nasal passages of animals in all exposure groups were observed. The primary lesion reported was atrophy of the olfactory epithelium and of the olfactory nerve fibres with or without focal respiratory metaplasia, and dilatation, hypertrophy and hyperplasia of the Bowman's gland. Abnormalities of the lungs were observed in the lungs of the majority of animals at 100, 150 and 200 ppm. These findings included decreased eosinophilia of the bronchial epithelium with focal crowding of non-ciliated cells in the bronchioles. No abnormalities of the lungs were observed in animals at 50 ppm. No abnormalities were observed in the livers of animals at 50 or 100 ppm. Abnormal liver histopathology (inflammation, fibrosis and hepatocyte loss) was observed after 13 weeks in 2 males at 200 ppm and in 5 females at 150 ppm and all surviving females at 200 ppm. The severity of these liver effects was more marked in females than in males.

BrdU labelling showed no increase in cell proliferation in the liver cells at any exposure level. An increase in the labelling index of the respiratory epithelium Clara cells was observed at weeks 2 and 5 in some animals at 150 and 200 ppm. No increase was observed at week 13. No such effects were observed at 100 ppm. Cell proliferation in nasal tissue was not investigated. Overall, a NOAEC was not evident from this study, with nasal epithelium damage being seen at 50 ppm, the lowest exposure level used.

In a study for which only an abstract is available, groups of $B6C3F_1$ mice (number per group not specified) were exposed to 62.5 to 500 ppm styrene for 90 days (exposure regimen not detailed) (Roycroft *et al,* 1992). The abstract reports the following observations (no further details are available). No treatment-related deaths were reported in females but treatment-related deaths were observed in males at 250 and 500 ppm (incidences got given). No clinical signs of toxicity were observed at any exposure. Body weight gain was reduced in all exposure groups. Relative liver weights were increased in mice at the two highest exposures and relative lung weights were increased in all exposure groups. "Liver enzyme activities" were increased in mice at 125 ppm and above. Other haematological and biochemical analysis were unaffected. Exposure-related histopathological findings comprised centrilobular necrosis/cytomegaly/karyomegaly in the liver; forestomach hyperplasia and ulcer, mineralisation of the heart; degeneration of the larynx; bronchiolar regeneration and inflammation; and cortical tubular necrosis in the kidney. An exposure-related increase in oestrus cycle was also reported. It is not clear from the abstract whether or not all exposure levels produced such effects.

In a short-term repeated exposure study, groups of CD-1 and $B6C3F_1$ mice (20 males and 20 females per strain) were exposed whole-body for 6 hours/day, 5 days/week for 2 weeks to 0, 15, 60, 250 or 500 ppm styrene (Kenny, 1992 and Cruzan et al, 1997). Signs of toxicity and bodyweight were recorded. A histopathological examination was performed on lungs, liver, kidneys and any tissue found to be macroscopically abnormal. The nasal passages were not examined. Toxic effects were noted in both strains, with many treatment-related deaths at 250 and 500 ppm. During exposure signs of irritation (not specified) were observed

in both strains at all exposure concentrations. Also during exposure, general signs of toxicity (lethargy, prone posture) were observed in both strains at the highest concentration only. Statistically significant increases in liver and kidney weights occurred at 500 ppm (quantified data not reported). In groups of animals of both strains exposed to 250 and 500 ppm, macroscopic liver changes in centrilobular hepatocyte necrosis were observed. Treatment-related siderocytes and/or prominent mitotic figures were seen microscopically in the liver in some animals from both strains at 60 ppm and above. Leucocytosis and single cell necrosis in the bronchiolar epithelium were found in the lungs at 250 and 500 ppm. In $B6C3F_1$ mice there was an increased incidence of perivascular inflammatory cells in the lungs at 60 ppm and above. The only microscopic findings in the kidney were minimal tubular necrosis, swollen epithelial cells or eosinophilic granular contents of 500 ppm in CD-1 mice only. There were no treatment-related findings in the organs examined in animals of either strain at 15 ppm.

In a study specifically conducted to investigate the potential effects of styrene on sperm morphology (Salomaa *et al*., 1985), groups of 9-14 mice were exposed whole body 6h/day for 5 days to 0, 150 or 300 ppm styrene. Two additional groups of animals given intraperitoneally 40 or 80 mg/kg/day cyclophosphamide, were used as positive control animals. The mice (groups of 4-7 animals) were then sacrificed 3 and 5 weeks after the beginning of the exposure to investigate potential effects on spermatids and on late spermatogonia/early spermatocytes, respectively. Head-shape morphology was examined for 500 sperms per mouse obtained from the cauda epididymides. No statistical significant increase in the frequency of abnormal sperm heads was detected 3 or 5 weeks after the beginning of the exposure. Cyclophosphamide induced, as expected, a positive response. Negative results were also obtained in this study in groups of 9-14 mice given intraperitoneally 175, 350 or 700 mg/kg/day styrene in olive oil for 5 days.

Studies in mice investigating specific organ toxicity: liver

Groups of at least 20 male and 20 female $B6C3F_1$ mice were exposed whole-body to 0, 125, 250 or 500 ppm styrene for 6 hours/day for 14 days (Morgan *et al,* 1993a). Mortality data were reported for the total mice exposed to styrene but only 10 per sex per group were included in the main element of the study which concentrated on effects on the liver. Remaining animals were included in a study of the immune system by Corsini *et al* (1994), summarised below. Hepatotoxicity was assessed by liver weight and histopathology results. Styrene-related deaths occurred in the 250 and 500 ppm groups, mainly after only 1or 2 exposures at about 12 hours post-exposure. In male mice, 8 or 27 died at 500 ppm and 11 of 25 died at 250 ppm. In female mice deaths (6 of 65) only occurred at 250 ppm. There was no significant effect on bodyweight. Severe histopathological effects were found in the livers of decedents. These included degeneration of necrosis of hepatocytes. In survivors, necropsied at 14 days, hepatic lesions included pigmentation, focal necrosis and inflammation. More severe histopathological changes were observed at 500 ppm than at 250 ppm; the main lesion was karyomegaly and cytoplasmic basophilia in centrilobular hepatocytes. No liver effects were seen at 125 ppm.

In a study comparing the hepatotoxicity of styrene in different strains of mice (DBA/2, $B6C3F_1$, C57BL/6 and Swiss strains), groups of 20 mice/sex/dose/strain were exposed to 0, 125, 250 or 500 ppm styrene for 6 hours/day for 4 days (Morgan *et al,* 1993c). Histopathological changes and liver weights were evaluated as indicators of hepatotoxicity. Styrene oxide levels in blood were measured as an indictor of styrene uptake and metabolism. The results demonstrated that the DBA/2 strain of mouse was much less susceptible to hepatotoxicity caused by styrene than the other strains tested, with no deaths and only “slight” signs of hepatotoxicity being reported at exposure concentrations of up to 500 ppm. In the $B6C3F_1$ strain, deaths (14/20 male mice died at 250 ppm and 3/20 at 500

ppm) and hepatotoxicity (increased liver weight and necrosis) were observed at 250 and 500 ppm. Strain C57BL/6 showed a similar response to $B6C3F_1$ of which it is a parental strain. Swiss mice exhibited a dose-dependent increase in mortality, liver weights and liver necrosis from 250 ppm (0 males and 3 females died at 250 ppm and 10 males and 8 females died at 500 ppm). The severity of the increased liver weights and necrosis was intermediate to the effects observed in $B6C3F_1$/C57BL strains of mice and the DBA/2 strain. The relative levels of blood styrene-7, 8-oxide metabolite did not follow the same order in the different strains and therefore did not correlate with the strain differences in evident hepatotoxicity.

In a study designed to investigate mouse strain and gender difference in susceptibility to hepatotoxicity following repeated exposure to styrene, groups of male and female $B6C3F_1$ and Swiss mice (total numbers not reported) were exposed nose only to 0, 150 or 200 ppm styrene vapour (99.9% purity) for 6 hours/day, 5 days/week for up to 2 weeks (Morgan *et al*, 1995). In the mouse, metabolism is not saturated at such concentrations (see section 4.1.2.1). Following the 2^{nd}, 3^{rd}, 5^{th} and 10^{th} exposures, changes in body weight, liver weight, serum alanine transferase (ALT), sorbitol dehydrogenase (SDH), total liver glutathione (GSH) and liver histopathology were determined in groups of 6 animals/sex/strain.

One female Swiss mouse was found dead after 4 exposures to 200 ppm. Necropsy revealed evidence of centrolobular hepatocellular necrosis. No male Swiss mice or $B6C3F_1$ mice of either sex died during treatment. Body weights and liver weights were similar in all animals. ALT and SDH levels were massively increased in female $B6C3F_1$ mice after 3 exposures to 200 ppm. Levels were only “slightly” elevated after 5 exposures and had returned to control levels after 10 exposures. Levels in all other test animals were comparable with controls throughout the study period. A dose related decrease in hepatic GSH levels in both sexes of both strains was observed at all time points in both exposure groups. The decrease was greatest (60-70%) in female $B6C3F_1$ mice at 200 ppm at each time point. Histopathological examination revealed a dose related increased incidence of degenerative and coagulative necrosis of centrilobular hepatocytes in female $B6C3F_1$ mice after 2, 3 and 5 exposures. Following 10 exposures the lesions were absent but inflammation was still present. Degeneration was also observed in 1 male $B6C3F_1$ mouse after 3 exposures to 200 ppm but not in any other male at other time points. Apart from in the decedent, no lesions were observed in Swiss mice of either sex at any exposure concentration or time point.

The results of this study indicate that $B6C3F_1$ mice are more susceptible to styrene-induced hepatoxicity than Swiss mice, and that female mice of this strain are more sensitive than the males. The possible reason for these differences (intra-species and between sexes) is probably a result of different rates of metabolism of styrene to styrene oxide, as indicated by differences in GSH depletion rates.

As part of an excretion study (see section 4.1.2.1.2), increased mortality (25%), elevated serum ALT levels (7-fold increase over controls) and evidence of hepatoxicity (severe centrolobular congestion, followed by acute necrosis) were observed in $B6C3F_1$ mice exposed to 250 ppm styrene via whole body exposures for 6 hours/day for 1-5 days (Sumner *et al,* 1997). In CD-1 mice the liver effects observed were quantitatively similar to those observed in $B6C3F_1$ mice but were of a less severe nature and did not appear until later in the exposure period. In recovery animals of both strains, complete regeneration of the livers was found on day 5 of recovery.

A series of studies has been conducted in the mouse investigating potential hepatoxicity following single and repeated exposures to styrene (Mahler *et al,* 1999). Single exposure data and further study details are reported in section 4.1.2.2.1. For the repeated exposure study, groups of 20 animals were exposed to 500 ppm styrene for 6 hours per day for 14 days; some animals were then re-exposed 10 days later to the same concentration of styrene. A satellite group of animals was sacrificed on day 5 of exposure and the liver examined. On day 14 of exposure or immediately following the re-exposure blood enzyme

levels were measured. Animals were sacrificed and microscopic examinations conducted on days 14 and 22 post-exposure, or after the re-exposure.

Two animals were found dead on the day after receiving the second exposure to styrene. No further deaths occurred throughout the remaining 12 days of exposure. No cause of death was reported. In the exposed group examined on day 5 post-exposure, liver weights were statistically significantly increased (by 26%) compared with controls and microscopic analysis showed discrete areas of centrilobular hepatocellular necrosis with associated borders of inflammatory cells (the severity and incidence of the findings was reported by the authors to be greater than that observed in the single exposure study, see section 4.1.2.2.1). Numerous hepatocytes in mitosis were also present. Also, at this time point there was a 15-fold increase in the BrdU labelling index compared with control levels. Labelled hepatocytes and inflammatory cells were numerous and randomly distributed. In the group examined at day 14, liver weights and BrdU labelling indices remained statistically significantly increased compared with controls (liver weight by 28% BrdU labelling index by 8-fold), whereas serum enzyme levels were comparable with controls. Microscopic examination revealed complete regeneration of necrotic centrolobular regions, with regenerated hepatocytes having basophilic cytoplasm and enlarged nuclei. In the group examined on day 22, prior to re-exposure, microscopic findings were similar to those reported above for day 14. After re-exposure no animals died but microscopic examination revealed evidence of acute necrosis of the centrolobular hepatocytes with enlarged nuclei and significantly elevated SDH and ALT levels.

Studies in mice investigating specific organ toxicity: respiratory tract

In an unpublished study specifically designed to investigate the effects of styrene exposure on the nasal tissue, groups of 10 male mice were exposed whole body to styrene vapour (99.9% purity) concentrations of 0, 40 or 160 ppm for 6 hours/day for 3 days (Foster, 1999b). Additional groups of males (10/group) were administered orally 200mg/kg of the cytochrome P450 2F2 inhibitor 5-phenyl-1-pentyne (5-P-1-P) prior to styrene exposure. Body weights and clinical signs of toxicity were apparently assessed throughout the study. Animals were sacrificed 18 hours after the last styrene exposure and nasal passages removed for histopathological examination.

Body weights were similar to controls throughout the study and no clinical signs of toxicity were reported. No treatment related macroscopic findings were observed in any of the exposed mice. No treatment related microscopic findings were observed in any of the control animals (including those administered 5-P-1-P but not styrene) or any of those exposed to 40 ppm styrene (including those administered 5-P-1-P) or in animals administered 5-P-1-P and exposed to 160 ppm styrene. In animals exposed to 160 ppm styrene alone, localized atrophy of the olfactory epithelium in the dorsal regions of the nasal passages and focally decreased numbers of Bowman's glands in these regions was observed. No changes in other parts of the nasal passages were apparent. The cytochrome P450 inhibition studies here and those in Green (1999d) (see below) both demonstrate a necessity for cytochrome P450-catalysed metabolism of styrene in the mouse respiratory tract before toxicity is expressed.

In an unpublished study specifically designed to investigate the effects of styrene exposure on the lungs, groups of male and female CD1 mice (5/sex/group) were exposed whole body to styrene vapour (99.9% purity) concentrations of 0, 40 or 160 ppm, for 6 hours/day, 5 days/week for 2 weeks (Green, 1999a). Groups of 5 animals/sex were sacrificed 17 hours after the 1st, 5th, 6th and 10th exposure(s), lungs removed for histopathological examination and quantitation of cell division rates (3 days prior to sacrifice each animal was fitted with a BrdU mini pump). Body weights and signs of toxicity were not reported. No treatment related macroscopic findings were observed in any of the exposed mice. Following repeated

exposure to styrene (both concentrations) treatment related focal loss of apical cytoplasm of on ciliated cells, principally in the terminal bronchioles, was observed. The magnitude of this finding varied between individual animals, with females appearing to be slightly more sensitive than males. Crowding of non ciliated cells throughout the bronchiolar tree was observed. There was no obvious dose related dependent increase in the cell labelling indices of the terminal bronchioles of animals, with the largest increase (3-4 fold) being reported in animals exposed to 160 ppm.

In another unpublished study by the same authors, again designed to investigate the effects of styrene exposure on the lungs, groups of male CD1 mice (5/sex/group) were exposed whole body to styrene vapour (99.9% purity) concentrations of 0, 40 or 160 ppm for 6 hours/day for 4 days (Green, 1999d). Body weights and clinical signs of toxicity were monitored throughout the study. Groups of 10 animals/sex were sacrificed 17 hours after each exposure and the lungs were removed for histopathological examination and quantitation of cell division rates (3 days prior to sacrifice each animal was fitted with a BrdU mini pump). In a second part to this study, as with that of Foster *et al,* (1999), additional groups of 10 animals/sex were administered orally 0 or 200 mg/kg of the cytochrome P450 inhibitor 5-P-1-P.

Body weights and clinical signs for all styrene exposed animals were normal and comparable with controls. On microscopic analysis, evidence of necrosis and loss of cells from the large bronchioles (thought to be Clara cells by the authors) was observed in animals examined after only one exposure to 40 ppm. At later time points (after 2 and 4 exposures), changes in the appearance of the Clara cells and of their organisation within the epithelium remained prominent. No information was given on histopathological findings with 160 ppm. BrdU studies demonstrated a statistically significant increase in pulmonary cell replication in the epithelium of the terminal and large bronchioles after 3 and 4 days of exposure to both 40 and 160 ppm styrene. There was no evidence of increased cell replication in the alveolar region. Pre-treatment with 5-P-1-P prevented the styrene induced increases in bronchiolar epithelium cell replication, indicating the importance of P450-catalysed metabolism of styrene to styrene oxide in the process by which styrene affects the respiratory epithelium in mice.

In a study specifically designed to investigate the effects of styrene exposure on the lungs,groups of female CD-1 mice (35/group) were exposed whole body to styrene vapour (99.9% purity) concentrations of 0, 40 or 160 ppm (0, 172, 688 mg/m^3) for 6 hours/day, for 1 to 5 days or for 4 weeks (5 days/week) (BASF, 2001a; Gamer et al., 2004). Malondialdehyde and 8-hydroxy guanosine (8-OHdG) levels and superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase activities were determined in the lung homogenates from each group in order to assess the potential of styrene inhalation exposure to produce oxidative stress in the lungs. Lung lavage was performed on a further 5 animals per group from each exposure duration and cytological and humoral analyses conducted. The levels of CC16 (clara cell specific protein) in the serum and lavage fluid were also measured. Glutathione levels in homogenates and lavage fluid were determined after 20 exposures only. At necropsy, the lungs, together with larnyx and trachea were removed and samples of the lung tissues from the control and high dose groups were examined by electron microscopy.

No consistent concentration- and time- related changes were observed in enzyme activity (catalase, superoxide dismutase, GSH reductase and GSH peroxidase) in the lung homogenates. The concentration of 8-OHdG was lowered in all the exposed groups except for the 160 ppm animals after 20 exposures. Statistically significant increases in lung lavage alkaline phosphatase (AP) were noted in the 160 ppm group at all exposure durations and in lung lavage LDH levels after 1 and 5 exposures. In animals at 160 ppm only, increases in malondialdehyde levels and in γ-glutamyl transferase levels were also reported and the glutathione concentration was significantly decreased in the homogenates. The serum levels

of CC16 were significantly decreased in animals at 40 and 160 ppm; the effect was most pronounced after 1 exposure. Similarly, a marked decrease in CC16 protein in lavage fluid was observed at the same concentrations following 1 and 5 exposures. Histopathological examination of the lungs showed epithelial desquamation, ballooning and vacuolation in the terminal bronchioles, large and medium airways in animals at 160 ppm after 1 exposure. Cellular crowding, expressed as an irregular epithelial lining (which is indicative of very early hyperplasia), and a reduction of apical blebs and secretory granules in Clara cells were seen at the same concentration (i.e. 160 ppm) after 5 and 20 exposures. Electron microscopy of lung tissue further revealed degenerative lesions (vacuolar cell degeneration and cell necrosis) or slightly different structures (absence of electron dense oval granules) in the Clara cells of the 160 ppm animals.

The results of this study suggest that Clara cell destruction may be the underlying mechanism for the pneumotoxic effects seen in mice exposed to styrene. Even though, slight glutathione depletion was observed at 160 ppm after 20 exposures, the other parameters did not indicate evidence of oxidative stress. Malondialdehyde (an indicator of lipid peroxidation) was only slightly elevated after 1 exposure at 160 ppm but decreased after 20 exposures at both 40 and 160 ppm. This lack of consistency in the observations for the oxidative stress parameters did not support previous conclusions about the role of oxidative stress in styrene-induced lung damage in mice. On the other hand, Clara cell toxicity was indicated by marked decreases in CC16 protein concentrations in both lavage fluid and blood serum, and by the microscopic observation of degenerative lesions. The irregular epithelial lining observed after 5 or 20 exposures was considered to represent regenerative hyperplasia. Clara cell toxicity and (regenerative) cell proliferation respectively were also indicated by increased γ-glutamyl transferase and AP activities noted at 40 ppm after 5 or 20 exposures and at 160 ppm after 1, 5 or 20 exposures. Overall, the findings of this study suggest that early biochemical changes in Clara cells (observed from 40 ppm), sustained cell damage and regenerative cell proliferation may be the mode of action for the carcinogenic effect of styrene in mice.

Studies investigating specific organ/tissue toxicity: haemopoietic stem cells

In a study designed to investigate the effects of styrene on haemopoietic stem cells, groups of female C57BL/DBA mice (group size not specified) were exposed for 6 hours/day, 5 days/week to styrene vapour concentrations of 0, 200 or 440 ppm for 8 weeks (Seidel *et al,* 1990). It is not reported if exposures were nose only or whole body. Due to some deaths in animals at 440 ppm by day 2 the exposure concentration was reduced to 330 ppm for the remainder of the study. At 4 and 8 weeks, blood and bone marrow samples were analysed for total erythrocyte, granulocyte, WBC, spleen colony forming units (CFU-S), erythroid burst forming units (BFU-E), erthyroid colony forming units (CFU-E) and granulocyte committed stem cells (CFU-C) numbers.

Results were not reported in detail. It was stated that no effects on red blood cell, granulocyte, CFU-S or CFU-C numbers were observed. Lymphocyte, BFU-E and CFU-E numbers varied considerably in all groups (including controls), but generally counts in cells of treated animals (both exposure groups) were reduced by 40 to 80% compared with controls. However, given the high variability in the colony numbers and the general lack of information in the report, no conclusions regarding the toxicological significance of styrene on the haematopoetic stem cells can be made from these study data.

Studies in mice investigating specific organ/tissue toxicity: immune system

As part of the Morgan *et al* (1993a) study described above, the same group of researchers subsequently reported a study of immunotoxicity involving groups of female B6C3F_1 mice

exposed to 0, 125, 250 or 500 ppm styrene for 6 hours/day for 14 days (Corsini *et al,* 1994). Animals were sacrificed 24 hours later. Kidney, liver, thymus, spleen and lungs were weighed and bone marrow cells obtained from the femurs. Results were presented for 6 or 7 animals per group. Mortality rates are reported above (Morgan *et al*, 1993a). The concentrations of styrene inhaled were relatively high for this strain and sex; deaths and liver toxicity were observed at 250 and 500 ppm in the related study. Body weight and relative lung weight were not altered by treatment. Relative liver and kidney weights were increased by 12-25% at 250 and 500 ppm. Relative thymus weight was decreased by 54% and spleen weight by 14% at 500 ppm only. Spleen cellularity was decreased by 21%, 14% and 46% in the 125, 250 and 500 ppm groups respectively. No significant effects were noted on relative numbers of B and T lymphocytes and natural killer cell activity. There were no effects on bone marrow cells.

Summary of repeated inhalation exposure studies in mice

As with acute toxicity, the mouse as a species is peculiarly sensitive to toxicity arising from repeated inhalation exposure to styrene, although there is some variation between different strains. Three well characterised traget sites have been identified: the lung, the nasal epithelium and the liver.

In relation to the lung, a NOAEC has not been identified, with damage to the lung epithelium (hyperplasia in the terminal bronchioles, focal bronchiolar-alveolar hyperplasia, fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct) having been seen on long-term exposure of mice to 20 ppm, the lowest exposure concentration tested. Nasal epithelium damage (respiratory metaplasia of the olfactory epithelium, hyperplasia and hypertrophy of the Bowman's gland, and atrophy of the olfactory nerve fibres) also appeared at 20 ppm (the lowest exposure level tested) and above. Work with the cytochrome P450 inhibitor 5-P-1-P has shown that metabolic activation of styrene to styrene oxide and other reactive metabolites (e.g the downstream metabolites of 4-vinylphenol) and subsequent detoxification of styrene oxide are crucial elements of the mouse respiratory tract toxic response. In this context it is important to consider that cytochrome P450-catalysed metabolic activation of styrene in human respiratory tract tissue appears to be negligible (see toxicokinetic section). Besides these metabolic differences, there are also significant anatomic and histological differences in the respiratory tract of mice and humans. Hence, it can be concluded that these respiratory tract findings in mice reflect a toxic process that will not occur to any significant extent in humans at relevant levels of exposure.

Exposures in the range 150-250 ppm have also produced fatal hepatotoxicity in mice. Again, it appears that the metabolic activation of styrene to styrene oxide is a crucial stage in the hepatotoxic response. In this context, it is evident that the toxicokinetic information available suggests that the mouse would be more susceptible than humans to styrene-induced hepatotoxicity. This is borne out by the observation of mouse fatalities at exposure levels to which humans have been regularly exposed in the past, with no reports of hepatotoxicity or death.

Overall, the data suggests that the mouse is a poor model for predicting the effects of repeated inhalation exposure to styrene in humans.

Other Species

As part of the above reported rat study the effects of repeated inhalation exposures to styrene on guinea-pigs, albino rabbits and monkeys were also investigated by Spencer *et al* (1942) and Wolf *et al* (1956). Groups of 24, 94 and 12 guinea pigs were exposed to 650, 1449 or 2139 ppm styrene, respectively for 7-8 hours/day 5 days/week for 21 weeks.

Groups of 12 or 2 rabbits were exposed to 1449 or 2139 ppm styrene, respectively for 7-8 hours/day, 5 days/week for 25 weeks. A group of 4 monkeys (2 male and 2 female) was exposed to 1449 ppm for 7-8 hours/day 5 days/week, males for 7 months and females for 12 months. During exposure regular body weight checks and observations of clinical toxicity were made. Frequent blood samples from representative animals were also taken for haematological analysis (timings of samplings not reported). At the end of the exposure period gross and microscopic examinations of an extensive range of tissue and organs was conducted.

No effects were noted in guinea pigs exposed to 650 ppm. At 1449 ppm 10% of the guinea pigs died after the first few exposures. Necropsy of the decedents revealed pronounced irritation of the lung (congestion, haemorrhage, oedema, exudation and inflammation). No other treatment related deaths occurred. In surviving animals signs of irritant effects on the eyes and nasal mucosa and reduced weight gain (not quantified) were noted at 1449 and 2139 ppm. Animals at these exposure concentrations also appeared dirty and unkempt. At necropsy, no abnormal lesions, changes in organ weights or haematological changes were noted at any dose level.

In rabbits and monkeys no treatment related deaths, no changes in body weight gain, no adverse clinical observations, no signs of irritation, no adverse gross or histopathological findings and no changes in haematology were observed in animals at the exposure concentrations tested.

The effect of inhalation exposure of styrene on neurochemicals in the brain of rabbits has been studied (Romanelli *et al,* 1986). Groups of eight New Zealand white rabbits were exposed whole body to 750 ppm of styrene for 12 hours/day for 7 days. Controls were exposed to clean air. Twelve hours after the final exposure the brains were dissected and assessed for dopamine, homovanillic acid (HVA) and norepinephrine. Mean dopamine levels were lower (by ~25-30%) and mean HVA levels were higher (by ~20-50%) in the striatum and tuberoinfundibular system in styrene exposed compared to control animals; no differences were observed in norepinephrine levels. The toxicological significance of these findings is unknown.

Groups of 6 male guinea pigs were exposed, whole-body, to 0 or 1000 ppm (4330 mg/m^3) styrene vapour, 6 hours/day for 5 days (Lataye *et al*, 2003). At regular intervals during the exposure period urine samples were taken for measurement of urinary metabolites of styrene. Cochlear function was tested before, during the 5 days of exposure, and for up to 4 weeks post-exposure using cubic distortion product otoacoustic emissions (DPOAE) recorded from the external ear canal in anaesthetized animals. A satellite group of animals was anaesthetized immediately after the last styrene exposure, killed and subjected to blood sampling for analysis of styrene levels. An additional group of 6 male guinea pigs exposed, whole-body, to 1000 ppm styrene vapour, 6 hours/day for 5 days was sacrificed four weeks after the last exposure for removal and analysis of the aural bones and tissues.

The bodyweights of animals were recorded weekly – no differences from controls were noted during the course of this study. No significant changes in the cochlear function were observed between styrene-exposed animals and the control animals. Blood styrene levels on completion of 5 days of styrene exposure were 4.9 μg/g. Urinary hippuric acid levels at 3 and 4 days post-exposure were around 8 g/g creatinine.

Summary of Repeated Inhalation Exposures Studies in Animals

A variety of repeated inhalation exposure studies in different animal species are available. However, among these species, the rat and mouse have been the most extensively investigated. Four well characterised target sites of toxicity have been identified: the nasal

epithelium (in rats and mice), the lung (in the mouse), the liver (in the mouse) and the ear (in the rat).

In relation to nasal epithelium damage, a NOAEC has not been identified, with chronic inflammatory changes (mild in degree and confined to the olfactory epithelium with no effects on Bowman's glands or olfactory nerve fibres), having been seen in the rat with long term daily exposure to 50 ppm styrene, the lowest concentration explored in a 2-year study. Nasal toxicity has also been reported in mice. In a similar 2-year study, respiratory metaplasia of the olfactory epithelium, hyperplasia and hypertrophy of the Bowman's gland and atrophy of the olfactory nerve fibres were observed starting from 20 ppm, the lowest concentration tested. It is clear that the nasal lesions induced by styrene exposure are much more severe in the mouse compared to the rat. Over the years a number of investigative studies have been undertaken to characterise and explain these species differences and to investigate the relevance of these findings to humans. Results of these investigations have shown that:

1) Nasal toxicity in the mouse is reduced considerably by pre-treating the animals with 5-phenyl-1-pentyne (5-P-1-P), a specific inhibitor of cytochrome P450 2F2 (Green*,* 1999c; Green *et al*., 2001b).

2) Metabolism of styrene to styrene oxide (to both R- and S-isomers) by olfactory and respiratory microsomes is similar in the mouse and rat (approximately 7 nmol SO/min/mg protein). Examination of nine human nasal samples (8 as S9 fractions and 1 as a microsomal fraction) failed to detect metabolism to styrene oxide (detection limit 0.04 nmol SO/min/mg protein which indicates a difference between rodents and humans in the production of SO of at least 2 orders of magnitude) (Green*,* 1999c; Green *et al*., 2001b).

3) Detoxification of styrene oxide is more efficient in human and rat nasal tissues as compared with mice. For example while human nasal tissue does not appear to possess any or very little P450 (activation) capacity, it does possess epoxide hydrolase activity, which is about 3-fold higher than that measured in mouse nasal tissues. The activity of epoxide hydrolase for both of the isomers in rat nasal tissue is higher (by a factor of approximately 10) than in the mouse, especially in the olfactory epithelium. Thus while both mouse and rat nasal tissues appear to be equally efficient at "activating" styrene to styrene oxide, the rat nasal tissue is more efficient at deactivating/detoxifying styrene oxide. Glutathione conjugation appears to be an important detoxification pathway in both rat and mouse nasal tissue with glutathione-S-transferase activity in rat nasal tissue being about 3-fold higher than the mouse (Green*,* 2000c; Green *et al*., 2001b).

4) Differences in the distribution of CYP2F2 in mouse and rat nasal tissues explain the histopathological differences seen in the mouse and rat. For example the mouse shows high levels of CYP2F2 expression in the apical cytoplasm of the olfactory tissue with low to moderate expression in some basal cells and in Bowman's glands. All of these cells show histopathological damage in the mouse after styrene exposure. Conversely in the rat such cells showed low expression of the metabolizing enzymes and no tissue damage (Green*,* 1999c, Green *et al.,* 2001b).

5) 4-Vinylphenol (4-VP), a metabolite of styrene, that rapidly undergoes further metabolic degradation, has been shown to be pneumotoxic to metabolically competent cells such as Clara cells (Kaufmann *et al*, unpublished, 2004) and presumably to nasal epithelial cells which also contain CYP2F2. As inhibition of CYP2F2 activity inhibits 4-VP toxicity in the lung (Carlson, 2002b), it appears that downstream metabolites of 4-VP rather than 4-VP itself are the cytotoxic species. Studies with rat, mouse and human lung microsomes have shown that the oxidative metabolites of 4-VP are ring hydroxylated and side-chain epoxide products of 4-VP and that the relative formation rates of these chemical species indicate that the mouse ability to produce these metabolites is greater than in the rat (14-79% of the mouse concentrations) and even greater than in humans (1.5-5% of the mouse concentrations) (Bartels et al, unpublished, 2004). A similar toxification of styrene via 4-VP and its

downstream metabolites may also be presumed to be mediated by CYP2F2 in the nasal tissue especially of mice.

6) Uptake of styrene by nasal tissue is enhanced by the ability of the tissue to metabolise styrene (Morris, 2000). The lack of the primary step of styrene metabolism in human nasal tissue serves as a further reason for dismissing the relevance to humans of these rodent nasal lesions.

7) Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which result in significant different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is likely that high levels will not be deposited in the olfactory area.

8) Human investigations have shown that exposure up to 77 ppm (8h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function (Dalton et al, 2003; see 'studies in humans', 'studies on olfaction').

Overall, it can be concluded that the differences in nasal toxicity between rat and mouse can be explained by the greater ability of the rat nasal epithelium to detoxify reactive metabolites of styrene formed *via* CYP2F2 metabolism. These reactive/toxic intermediates include styrene oxide and most probably the downstream metabolites of 4-VP. Detoxification of toxic species by epoxide hydrolase is 10-fold higher in the rat olfactory tissue as compared to the mouse while glutathione S-transferase activity is approximately 3-fold higher in the rat nasal tissue as compared to the mouse. It is important to consider that cytochrome P450-catalysed metabolic activation of styrene to styrene oxide in human nasal epithelial tissue appears to be negligible (see toxicokinetics section). Also, since uptake of styrene by nasal tissue is enhanced by the ability of the tissue to metabolise styrene, the lack of the primary step of styrene metabolism in human nasal tissue serves as a further reason for dismissing the relevance to humans of these rodent nasal lesions. Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which result in significant different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is likely that high levels will not be deposited in the olfactory area. Furthermore, human investigations have shown that exposure up to 77 ppm (8h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function, and in several human health surveys of workers repeatedly exposed to styrene up to approximately 700 ppm, no nasal lesions have been described (see RDT, human studies section). Hence, it can be concluded that rodent nasal epithelium damage induced by styrene is not of relevance in relation to the potential repeated dose effects of styrene in humans at relevant levels of exposure.

In relation to the lung, again, a NOAEC has not been identified, with damage to the lung epithelium (hyperplasia in the terminal bronchioles, focal bronchiolar-alveolar hyperplasia, fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct) having been seen on long-term exposure of mice to 20 ppm, the lowest exposure concentration tested in a 2-year study. Work with the cytochrome P450 inhibitor 5-P-1-P has shown that metabolic activation of styrene to styrene oxide and other reactive metabolites (e.g the downstream metabolites of 4-vinylphenol) and the subsequent detoxification of styrene oxide are crucial elements of this toxic response. This is supported by the observation that it is the metabolically active Clara cells that are the initial focus of damage. These non-ciliated bronchiolar epithelial cells are mainly involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium (Komaromy and Tigyi, 1988). Early biochemical changes, sustained cell damage and regenerative cell proliferation were observed in lung Clara cells of mice exposed to 40 and 160 ppm styrene for up to 4 weeks. In this context it is important to consider that cytochrome P450-catalysed metabolic activation of styrene in human lung tissue appears to be minimal (see toxicokinetic section) even though the two main P450 isoforms involved in styrene metabolism, CYP2E1 and CYP2F1, have been detected; that

the number of Clara cells in human lung is very low; and that their most important functions in human lung are shared by other cell types (e.g. the Type II cells). Hence, it can be concluded that these lung tissue findings in mice reflect a toxic response that will not occur to any significant extent in humans at relevant levels of exposure.

In relation to the liver, exposures in the range 150-250 ppm have produced fatal hepatotoxicity in mice. Again, it appears that the metabolic activation of styrene to styrene oxide is a crucial stage in the hepatotoxicity process. In this content, it is evident that the toxicokinetic information available suggests that the mouse would be more susceptible than humans to styrene-induced hepatotoxicity. This is borne out by the observation of mouse fatalities at exposure levels to which humans have been regularly exposed in the past, with no reports of hepatotoxicity or death.

In relation to effects on the ear, clear evidence of ototoxicity (both functional and histological has been seen in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks' duration. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tend to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm. Comparative studies using rats and guinea pigs exposed to 1000 ppm for 5 days indicate an obvious species difference as similar findings were not observed in guinea pigs. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. The underlying toxicological mechanism has not been clearly elucidated. This effect should be therefore regarded as of potential relevance to human health. The histological damage consists in the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irriversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organisation of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. On this basis, it is considered that the NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively are a more accurate starting point for risk characterisation than the NOAEC of 200 ppm for 13 weeks.

In one single non-standard investigation, rather minor effects on the number of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks have been reported. However, it is considered that, in the absence of any associated conventional histopathology and given the lack of information on what are the 'normal' levels of dopamine and glutathione in the retinal cells of rats, the rather minor morphometric and biochemical changes observed are unlikely to represent a toxic response.

Little information is available on the effects of repeated inhalation exposure to styrene in other experimental animal species. In relatively old studies, no significant toxicological effects were reported in guinea pigs, rabbits and monkeys repeatedly exposed to styrene at up to 650 ppm (in guinea pigs, toxicity was seen at 1449 ppm), 1449 ppm (in monkeys) or

2139 ppm (in rabbits). However, these studies did not investigate nasal epithelial tissue in detail.

Overall, the available inhalation repeated dose toxicity studies have identified ototoxicity as the most sensitive and relevant effect of styrene repeated inhalation exposure with NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively.

Oral

Rats

No deaths, clinical signs of toxicity or changes in body or liver weights were observed in albino rats administered 0, 250, 450 or 900 mg/kg styrene orally by gavage for 7 consecutive days, in a study designed primarily to investigate effects of MFOs and glutathione S-transferase activities (Das *et al,* 1981). MFO activity was induced, but there was a decrease in hepatic GSH and glutathione S-transferase activity.

In a study by Spencer *et al* (1942), 59 rats in total (numbers per group not specified) were administered 0, 100, 500, 1000 or 2000 mg/kg/day styrene by oral gavage once daily, 5 days/week for 28 days. No further study protocol details were reported. No deaths and no adverse toxicological changes (growth, pathological or haematological changes) were observed in any of the animals administered 100 mg/kg/day styrene. Five animals administered 500 mg/kg/day styrene survived to day 20. These animals had decreased weight gain (not quantified) throughout the study. At the end of the study duration no significant adverse pathological findings were noted in animals of this dose group, except for "slight" signs of local irritation in the oesophagus and stomach. No adverse haematological findings were observed in animals of this group.

A "few" doses of 1000 or 2000 mg/kg/day resulted in a pronounced irritation of the oesophagus and stomach which quickly resulted in death. The number of decedents and the timings of deaths were not reported.

In a study by Wolff *et al* (1956), groups of 10 female Wistar rats were administered 67, 133, 400 or 667 mg/kg/day styrene in olive oil by oral gavage, once daily for 5 days/week for 6 months. A group of 20 animals served as a control group and received vehicle only. Haematological examinations (total erythrocyte and leukocyte counts, haemoglobin content and differential white blood cell count) were made on selected animals after the 20, 40, 80 and 130th doses. Animals were observed regularly for mortality and clinical signs of toxicity. Surviving animals were sacrificed 18-22 hours after the last exposure. Gross pathological examination was conducted on the lungs, heart, kidneys, liver, spleen and testes, but there was no microscopic examination of tissues.

No deaths were reported. No adverse effects (clinical observations or haematology) were observed in animals administered 67 or 133 mg/kg/day styrene. In animals administered 400 mg/kg/day a "slight" depression in growth and "slight" reductions in liver and kidney weights compared with controls were reported (changes not quantified). In animals administered 667 mg/kg/day styrene, the same effects were observed but were more pronounced (again changes not quantified). No abnormal gross pathological findings were observed in any of the dose groups, but unfortunately histopathological observations were not made.

A 2 year continuous exposure study has been conducted in Sprague-Dawley derived rats (Beliles *et al*, 1985). Styrene was administered in drinking water at 0, 125 or 250 ppm. Consumption of styrene was only 14 (males) or 21 (females) mg/kg/day at the higher dose level. Forty males and 50 females in each treated group and 66 male and 84 female control

animals were examined twice weekly for signs of toxicity and body weight, food and water consumption, haematology and clinical chemistry investigations. At necropsy at termination, organ weights were determined and histopathology carried out on a large number of tissues. The only finding was a slight but statistically significant reduction in bodyweight compared to control, of 10% in females at 21mg/kg/day after 2 years. In the absence of other findings, it appears that styrene did not produce any clear evidence of toxicity in this study.

Information is available from a cancer bioassay in which groups of 50 male and 50 female Fischer 344 rats were treated with 1000 or 2000 mg/kg styrene by gavage for 5 days/week for 78 weeks, and a third group was given 500 mg/kg for 5 days/week for 103 weeks (NCI, 1979). All doses were given as a solution in corn oil and a control group of 40 males and 40 females received vehicle only. The animals were observed until week 104 when the experiment was terminated. All animals were subject to a full gross and microscopic examination of a range of organs, including the reproductive organs. A marked reduction in survival was noted at 2000 mg/kg, with only 6/5 (12%) of male rats surviving for 53 weeks and 7/50 (14%) female rats surviving at week 70. No effects on survival were noted at the other dose levels, with 88-94% of the animals surviving for at least 90 weeks. The only compound related lesion noted at autopsy was hepatic necrosis in animals given 2000 mg/kg. Overall, a NOAEL of 1000 mg/kg can be identified from this study.

A series of studies has been conducted in order to measure the potential hepatic and testicular toxicity of styrene in the Wistar rat (Srivastava *et al,* 1982 and 1989). In each study groups of 6 adult male rats (age not further specified) received 0, 200 or 400 mg/kg/day styrene (purity not specified) in groundnut oil by gavage for 6 days/week for 100 or 60 days, respectively. In the study of hepatic effects, no clinical signs of toxicity were observed in treated animals. Body weight gain and absolute and relative liver weights of treated animals were comparable with controls. Indications of minor enzymatic changes were reported at both dose levels. Histopathological examination revealed small areas of focal necrosis, with a few degenerative hepatocytes and inflammatory cells, in top dose animals only.

In the study of potential testicular toxicity, at necropsy one testis/animal was examined histopathologically and the other was homogenized for enzyme assays. No changes in sperm count, histopathology or enzyme analysis were observed in animals receiving 200 mg/kg/day styrene. In animals at 400 mg/kg/day a statistically significant decrease in epididymal sperm count (5.0 million spermatozoa compared with 8.0 million in controls) and marked histopathological changes in the testes (shrunken seminiferous tubules with damaged nuclei and an absence of sperm in the lumen of some tubules) were reported. Also in top dose animals the activities of the marker enzyme activities for testicular function (sorbitol dehydrogenase, acid phosphate) were statistically significantly decreased, but those of lactate dehydrogenase, γ-glutamyl transpeptidase, β-glucuronidase and glucose-6-phosphate were statistically significantly increased. The relevance of these testicular enzyme levels to testicular function is unclear. It is also unclear whether there were direct testicular effects or whether the effects were secondary to testicular edema. It is noted that the mesh-filtration of epididymal sperm was not an optimal method of specimen preparation and that the testicular fixation method used was suboptimal and may have led to artifacts. It is also noted that the finding of shrunken tubules without an effect on testis weight appears inconsistent.

More recently, a similar investigation was conducted by the same authors (Srivastava *et al*., 1992b). Groups of seven 1-day old male Wistar rats were orally dosed 0, 100 or 200 mg/kg/day styrene for 60 days. Throughout the dosing period there were no clinical signs of toxicity. On completion of treatment (PND 61), 6 animals were randomly selected from each group and killed. Testis and epididymides were removed and weighed; the activities of testicular enzymes were measured and epididymal spermatozoa counts were performed. In the 200 mg/kg group there was a statistically significant decrease in the absolute and

relative weight of the testis, and a statistically significant decrease in spermatozoa counts. Furthermore, in the 200 mg/kg group there were reductions in the activity of testicular sorbital dehyrogenase and acid phosphatase, and increases in the activity of lactate dehydrogenase, β-glucuronidase, glucose-6-phosphate dehydrogenase and γ-glutamyl transpeptidase; all results were statistically significant. There were no effects at 100 mg/kg/day. It is noted that the mesh-filtration of epididymal sperm was not an optimal method of specimen preparation.

In view of the methodological weaknesses of these studies, little weight should be put on their findings. It is also noted that in earlier repeated oral studies and in well conducted 2 year inhalation studies in rats at equivalent and higher doses than those used by Srivastava et al, no testicular changes or indications of any testicular effects were observed. Also, no effects on the testis and fertility parameters have been observed in a recent well-conducted OECD- and GLP-compliant rat inhalation 2-generation study with exposures up to 500 ppm (≈ 300 mg/kg/day) styrene. Therefore, despite these individual publications by the same authors reporting testicular damage, the weight of evidence indicates that styrene is not a testicular toxicant.

Several studies are available in which the effects of repeated oral administration of styrene on central nervous systems parameters in rats have been investigated; one study also incorporates an exploration of potential ototoxicity. A study is briefly described in which groups of 12 male albino rats received vehicle (saline) or 906mg/kg/day styrene for 15 days by gavage (Husain *et al*, 1980). Animals were killed on day 16 and brains removed and homogenised. Samples from half of the animals in each group were analysed for levels of dopamine, norepinephrine and serotonin; samples from the remaining 6 per group were analysed for activity of monoamine oxidase (MAO) and acetylcholinesterase enzymes and protein content. Relative to the control group, in the styrene-treated animals there was a statistically significant increase in norepinephrine and serotonin and the activity of MAO significantly decreased. No change in dopamine and acetylcholinesterase activity was reported.

There is another limited report of the effects of styrene on MAO activity following oral dosing in rats (Zaprianov and Bainova, 1979). Groups of six male rats (strain not indicated) received either 1200 mg/kg/day (~25% of the LD_{50}) styrene, 3200 mg/kg/day (again ~25% of the LD_{50}) ethanol in water or the two combined for 7 days; controls were stated to have been “non-treated”. MAO activity was investigated in serum, liver and brain (homogenates for the latter two) after treatment but the time of assessment is not clear. Three different substrates were used to assess MAO activity – dopamine, kynuramine and serotonin. No indication is given in the report on whether or not any signs of toxicity were seen during the study as may have been expected given the dose levels used. Brain MAO activity, assessed using dopamine and kynuramine, was reduced by styrene treatment as was liver MAO activity when using serotonin as substrate. When combined with ethanol, the reduction in MAO activity was more pronounced in the brain but the change measured by serotonin in the liver was reversed. However, there was appeared to be inconsistency between the levels of apparent change in activity and the degree of statistical significance. Overall, given the deficiencies in reporting and the high doses used no meaningful conclusions can be drawn from this report.

The effects of repeated oral administration of styrene on neurobehaviour and neurochemistry have been studied (Husain *et al,* 1986). Groups of 15 male Wistar rats received 100 or 200 mg/kg styrene in groundnut oil by oral gavage for 14 days. Controls received an equal volume of groundnut oil. Twenty-four hours after the last treatment the rats were monitored for spontaneous locomotor activity before and after an i.p. dose (2.5 mg/kg) of amphetamine. On days 2-4 after treatment the rats learning behaviour was examined by conditioned avoidance response (CAR; using a sound stimulus and electric shock). Following the CAR, the brains from six rats from each group were assessed for

noradrenaline, dopamine and serotonin levels. No clinical signs of toxicity were observed and there was no difference in spontaneous locomotor activity either with or without amphetamine treatment. Treatment with styrene increased the mean percentage CAR on the 3rd and 4th days after treatment equally at both dose levels. There was no difference in noradrenaline and dopamine levels between the treated and controls groups. No differences were observed in mean serotonin levels at 100 mg/kg but levels were elevated in the mid brain (50% greater than controls), hypothalamus (~80%) and hippocampus (60%) at 200 mg/kg. The changes in this study suggest that styrene induced a better avoidance response in rats but the relationship, if any, to the differences in serotonin levels is unclear as these were seen at only one dose level.

Wang (1998) and Wang *et al* (1998) studied the effect of orally administered styrene on auditory response and brain immunohistochemical staining in rats. Groups of six adult male Wistar rats were implanted with brain electrodes one week prior to styrene exposure. The groups then received either 400 or 800 mg/kg/day styrene in olive oil by oral gavage for 5 days/week for two weeks; controls received saline. Further groups of rats (6/group) received similar treatment but were used for immunohistochemical analysis. Audiometry was performed prior to styrene exposure, twice during exposure (after the first and second week) and then at 2, 4 and 6 weeks after the end of treatment. The evoked response measured was “middle latency auditory evoked potentials” (MAEPs). Responses were stimulated by exposure to 200 ms bursts of noise at 5Hz and an intensity of 90 dB SPL. The typical output response for MAEP in the alert rat was described as a double sine-like waveform with two “negative” troughs and two “positive” peaks. Measurements made were the latency (i.e. time between stimulus and measurement) for each trough and peak, the peak to peak interval and the amplitude. For immunohistochemistry, brains were taken from rats at the end of the first, second, third and fourth months after treatment and sectioned sagittally. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) in the cerebral cortex and hippocampus was performed using fluorescent probes and quantitation of fluorescent intensity made using scanning confocal microscopy.

It was stated that no effects on body weight gain were seen in treated animals compared to controls. Audiometry revealed no differences in any parameter prior to treatment with styrene. Following treatment with styrene at 800 mg/kg/day, in general, latency times for all MAEP troughs and peaks were increased compared to controls with increases beginning to appear towards the end of treatment and during the post-exposure period. Some increases in latency times were seen also at 400 mg/kg/day but these tended to be less than at 800 mg/kg/day and were more inconsistent, being seen at some time points but not other ones with no apparent pattern. Peak to peak intervals (a reflection of delays between the stimulated waveforms) were increased compared to control only at 800 mg/kg/day. No difference in amplitude of the response was seen between controls and either treated group. Immunofluorescent staining of GFAP was increased in intensity at 800 mg/kg/day compared to controls at all time points measured in the cerebral cortex and at 1, 2 and 3 months post-exposure in the hippocampus. Fluorescent intensity was also increased at 400 mg/kg/day, but less than at 800 mg/kg/day, at 2 and 4 months in the cerebral cortex and at 2 and 3 months in the hippocampus. Overall, effects on auditory evoked potentials were seen in this study, reflecting styrene ototoxicity. This was accompanied by increases in fluorescent staining of GFAP in selected areas of the rat brain. The functional significance of these latter changes, though, is uncertain.

The behavioural characteristics of young rats fed normal and low protein diets and treated with styrene have been investigated by Khanna *et al* (1994). Groups of 50 immature (21 days old) male Wistar rats were fed normal or low protein diets for 15 days. When the rats were 36 days old, each of the dietary groups was split into two, one group receiving 250 mg/kg/day of styrene in groundnut oil by oral gavage or oil alone until 51 days of age. On the day after the last styrene treatment (52 days old) the rats underwent behavioural studies.

Groups were investigated for foot shock induced aggressive (fighting) behaviour, amphetamine induced locomotor activity and brain biochemistry (measurement of norepinephrin, dopamine and serotonin); dopamine receptors in the corpus striatum and serotonin receptors in the frontal cortex were also measured. Styrene alone (with normal diet) produced a slight but non statistically significant reduction in mean body weight gain compared to controls but treatment in combination with low protein diet enhanced the reduction in body weight gain induced by the low protein diet. The mean incidence of aggressive behaviour was slightly increased by styrene alone compared to controls and significantly increased when styrene was administered to the low protein group. Amphetamine induced locomotor behaviour was unaffected by styrene treatment under either dietary conditions. Dopamine, but not norepinephrin or serotonin levels were decreased (by about 50%) by styrene treatment in normal dietary rats compared to controls but all three were reduced in the low protein group. Dopamine and serotonin receptor levels were the same in styrene only and control rats but both were elevated in the rats treated with styrene on low protein diets. Overall, no significant effects, apart from a reduction in brain dopamine levels, were seen in this study in immature rats treated with styrene and fed on a normal diet.

Impairment of serial spatial reversal learning has been observed in adult male Long-Evans rats which received 500 mg/kg/day styrene for 1, 3, 5 or 8 weeks (Bushnell, 1994). Reversal training began at 8, 10 or 32 weeks after treatment and involved learning to receive a food reward following stimulus and the pressing of levers. A reversal involved switching reward values between levers. The significance of these findings is unclear.

Agrawal *et al* (1982) administered styrene by gavage in peanut oil at 0, 200, 400 mg/kg/day for 90 days to groups of 6 male albino rats. The study was reported only in brief. Animals were killed and membrane preparations from brain corpus striata were used to test for dopamine receptor binding 24 hours after the last dose was administered. Relative to the controls, there was a significant increase in specific binding of ^{3}H-spiroperidol to dopamine receptors reported at both dose levels of styrene.

Mice

In a study designed to investigate the effects of styrene on the mouse lung following oral dosing, groups of 10 male CD-1 mice per group were orally administered doses of 0, 10, 100 or 200 mg/kg/day styrene (99% purity) in corn oil, daily for 5 consecutive days (Green, 1999b). Body weight and clinical observations were made throughout the study. Animals were sacrificed 24 hours after the last dose and the lungs removed for histopathological examination and quantitation of cell division rates (3 days prior to sacrifice each animal was fitted with a BrdU mini pump). No animals died during the study, body weights in treated animals were comparable with controls and no clinical signs of toxicity were observed in any animals. There were no treatment related macroscopic findings in the lungs of animals at any dose level. No microscopic findings were made in animals administered 10 or 100 mg/kg/day styrene. However 3/10 animals at 200 mg/kg/day presented signs or ‘minimal’ to ‘slight’ focal crowding of non-ciliated cells in the epithelium of the terminal bronchiole. There was no evidence of cellular necrosis or damage. There was no evidence of an increase in cell replication in the large bronchioles or alveolar regions of animals at any dose level, nor in the terminal bronchiole of animals administered 10 mg/kg/day styrene. However, in animals administered 100 or 200 mg/kg/day styrene there was a statistically significant and dose dependent increase in the frequency of S-phase cells in the terminal bronchioles, with levels being increased up to 5 fold in top dose animals compared with controls. The significance of this study is that it demonstrates that systematically available styrene can produce respiratory tract effect in mice.In a study designed to investigate the potential immunotoxicity of styrene, groups of 30 Swiss mice were administered very large doses of 0,

1000, 1500 or 2500 mg/kg/day styrene (20, 30 or 50 mg per animal and assuming an animal weighs 20g), by oral gavage, daily for 5 consecutive days (Dogra *et al,* 1989). A fourth group of animals was employed as a positive control group and was administered 75 mg/kg cyclophophamide via the intraperitoneal route. At day 2 post exposure 8 animals per group were sacrificed and haematological analyses and histopathological examinations (spleen, thymus, liver, kidney, adrenal glands, portal and regional lymph nodes and Peyers patches) conducted. The remaining animals (6/group) were employed in different assays of immunological function. However, from the results obtained and given the extremely high doses used in the study, no meaningful conclusions can be drawn from the results reported in this part of the study. Spleen weights were decreased in a dose responsive manner in all styrene treated animals by 34, 37 and 73% of controls, respectively. Non-dose related fluctuations in other organ weights were reported. No overt signs of toxicity were observed in the lymphoid organs at gross pathological examination or in any of the haematological parameters investigated. Histopathological examination on top dose animals only revealed evidence of depletion in the cellular population of the periarteriolar lymphoid sheath and of the peripheral lymphoid follicles of the spleen, without affecting the red pulp areas. No other treatment related adverse histopathological findings were observed.

In a study designed to investigate the effects of styrene on the testes and on sex hormone levels, groups of 7 prepubertal male C57/BL6 mice received 0, 5 or 50 μg/ml styrene dissolved in 0.005% aqueous ethanol as drinking water for 4 weeks (Takao *et al*, 2000). These styrene concentrations are estimated to be equivalent to exposures of around 1.2 and 12 mg/kg/day respectively (based on bodyweight and water consumption data provided in the study report). Vehicle controls as well as water-only controls were used. Animals were estimated to be 5 weeks old at the start of this study. Blood samples were taken at terminal kill for measurement of free testosterone, corticosterone and luteinizing hormone levels. The only organs taken for histological examination were the spleen and one testis from each animal. There were no further investigations. There were no significant differences in water consumption, bodyweight gain, testis or spleen weight between styrene-exposed and control animals. On completion of the 4 weeks of exposure, plasma levels of free testosterone were markedly decreased in the high exposure group compared to controls (from around 50 pg/ml in controls to around 5 pg/ml at 12 mg/kg/day styrene). No clear differences emerged in levels of corticosterone or luteinizing hormones. No macroscopic or microscopic alterations were observed in the organs investigated. Reduced levels of testosterone have been observed in animals exposed to 12 mg/kg/day styrene for 4 weeks in this study, but the full toxicological significance of this finding is unclear given the absence of histopathological findings in the testes. Interpretation is also made difficult by the limited range of examinations (observations were made at one single point), the absence of background information on the possible normal range of testosterone levels that might be expected in these young mice and the absence of such effects at such low dose levels in other well documented studies.

In early studies, deaths have been reported in mice following repeated oral administration of 215 mg/kg/day or above for up to 7 weeks (Stewart *et al,* 1968). One of 10 mice died at 215 mg/kg/day, increasing to 8/10 at 681 mg/kg/day.

As part of the NCI (1979) study reported in the carcinogenicity section, groups of 50 $B6C3F_1$ mice per sex were administered 150 or 300 mg/kg of styrene by gavage, as a solution in corn oil, five days a week for 78 weeks (NCI, 1979). The animals were then observed for a further 13 weeks. A control group of 20 males and 20 females was administered vehicle only. All animals were subject to a full gross and microscopic examination of a range of organs, including the reproductive organs.

A significant decrease in survival was noted in the male mice at 300 mg/kg, with 78% surviving the experimental period, as compared to 92% and 100% of the animals at 150

mg/kg and in the control group respectively. A slight but not statistically significant decrease in survival was noted in the female mice, with 76% and 80% of animals of the 300 and 150 mg/kg groups respectively surviving the experimental period, compared to 90% of the controls. A “slight” but dose related decrease in bodyweight gain was noted in the female animals. No other clinical signs were reported. At necropsy, hepatic necrosis was found in 4/49 high dose males and in 6/48 high dose females. . Based on increased mortality and hepatic necrosis at the top dose (300 mg/kg), a NOAEL of 150 mg/kg can be identified from this 78 week study.

Other species

A repeat dose study in dogs is available, in which groups of 4 Beagle dogs per sex were administered styrene in peanut oil by oral gavage at dose of 0, 200, 400 or 600 mg/kg/day, 7 days per week for up to 561 days; dogs in the 600 mg/kg group received peanut oil only on treatment days 318-469, after which they were returned to styrene dosing for the remainder of the study (Quast *et al,* 1979). Animals were observed daily for clinical signs of toxicity. Body weight and food consumption was monitored throughout the study. Haematological analyses, urinalyses and clinical chemistry parameters were evaluated prior the start of treatment and at intervals throughout the test period. Ophthalmogical evaluation and gross microscopic examination of a range of tissues was performed.

No deaths occurred and no treatment related clinical signs or effects on body weight were observed. The only treatment related effect reported was a dose related increased incidence of Heinz bodies and associated changes in red blood cells in male and females at 400 and/or 600 mg/kg/day, and sporadically in low dose females. Other haematological effects in both sexes at 600 mg/kg/day were statistically significant decreases, or a trend towards a decrease, in packed cell volume, red blood cell count, haemoglobin level and erythrocyte sedimentation rate; an increased incidence of anisocytosis and hypochromasia of the red blood cells was also seen in males only at 600 mg/kg. Associated minor liver changes were also seen: an increased amount of hemosiderin pigment in the reticuloendothelial cells in both sexes at 400 and 600 mg/kg and in a single female at 200 mg/kg, and increased numbers of hepatocellular intranuclear acidophilic crystalline inclusions in both sexes at 600 mg/kg. Upon cessation of treatment, the haematological effects in the 600 mg/kg group were reported to be rapidly cleared and blood parameters returned to normal. It is reported that there was no indication of any increase in red blood cell turnover or of bone marrow hyperplasia.

Overall, this study suggests that the only effects of repeated styrene exposure are on haematological parameters. Although a clear NOAEL cannot be identified, the effects seen at 200 mg/kg are minimal.

Summary of Repeated Oral Exposure Studies in Animals

Most of the available repeated oral exposure studies have been performed in rats. Few of these are of the quality, breadth and thoroughness required in standard regulatory test guidelines. Hence, most of the data do not facilitate the derivation of an overall NOAEL of the type normally sought in relation to a 90 day (or longer) exposure period. However, there is reliable information from a carcinogenicity bioassay in the rat, in which there was no evidence of treatment related toxicity in animals administered 1000 mg/kg/day for two years; a marked increase in mortality was evident at 2000 mg/kg/day. In another 2 year study, styrene did not produce any clear evidence of toxicity when administered in drinking water at a dose of 21 mg/kg/day, the highest dose level used. However it is noted that potential effects on the ear were not investigated in these studies. Ototoxicity, a clearly established effect of styrene in the rat, was seen in one study at 800 mg/kg/day (and perhaps, although

less convincingly, at 400 mg/kg/day), for 2 weeks. A range of different CNS related observations have also been made with repeated oral dosing in this range, but unfortunately each one stands in isolation and lacks a surrounding, well established and validated framework within which the results can be interpreted, and in some cases contradictory results have been reported in different studies. Overall, there is no convincing evidence of clear, styrene induced neurological lesions induced by repeated oral dosing in rats. Two studies by the same authors (Srivastava et al) have reported testicular damage in rats at 200 and 400 mg/kg/day styrene. It is noted that in earlier repeated oral studies and in well conducted 2 year inhalation studies in rats at equivalent and higher doses than those used by Srivastava et al, no testicular changes or indications of any testicular effects were observed. Also, no effects on the testis and fertility parameters have been observed in a recent well-conducted OECD- and GLP-compliant rat inhalation 2-generation study with exposures up to 500 ppm (≈ 300 mg/kg/day) styrene. Therefore, despite these individual publications by the same authors reporting testicular damage, the weight of evidence indicates that styrene is not a testicular toxicant.

In mice, the most reliable information comes from a cancer bioassay, in which increased mortality and hepatic necrosis were observed at the highest dose of 300 mg/kg/day, and a NOAEL of 150 mg/kg/day was identified. The one significant observation from the remaining studies is that of toxicity towards the lung epithelium, adding further support to the concept that the lung toxicity of styrene in mice, following oral and inhalation exposure, results from local metabolism of styrene to styrene oxide and other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol). The one report of styrene induced decrease in serum testosterone levels in young mice dosed with 12 mg/kg/day for 4 weeks is likely to be a chance finding.

A repeated oral study in dogs detected Heinz bodies in circulating erythrocytes (suggesting oxidative damage) at doses of 400 and 600 mg/kg/day, with minimal effects in females at 200 mg/kg.

Overall, in relation to repeated oral exposure, the NOAEL of 150 mg/kg/day identified from a 2 year cancer bioassay in the mouse should also be considered. But in extrapolation to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat.

Dermal

No studies are available, although low systematic toxicity would be predicted in most conventional experimental species with the possible exception of some strains of the mouse.

Summary of Repeated Dose Toxicity Studies in Animals

A variety of repeated inhalation exposure studies in different animal species are available. However, among these species, the rat and mouse have been the most extensively investigated. Four well characterised target sites of toxicity have been identified: the nasal epithelium (in rats and mice), the lung (in the mouse), the liver (in the mouse) and the ear (in the rat).

In relation to nasal epithelium damage, a NOAEC has not been identified, with chronic inflammatory changes (mild in degree and confined to the olfactory epithelium with no effects on Bowman's glands or olfactory nerve fibres), having been seen in the rat with long term daily exposure to 50 ppm styrene, the lowest concentration explored in a 2-year study. Nasal toxicity has also been reported in mice. In a similar 2-year study, respiratory metaplasia of the olfactory epithelium, hyperplasia and hypertrophy of the Bowman's gland and atrophy of

the olfactory nerve fibres were observed starting from 20 ppm, the lowest concentration tested. It is clear that the nasal lesions induced by styrene exposure are a lot more severe in the mouse compared to the rat. Over the years a number of investigative studies have been undertaken to characterise and explain these species differences and to investiagte the relevance of these findings to humans. The results of these investigations have shown that the differences in nasal toxicity between rat and mouse can be explained by the greater ability of the rat nasal epithelium to detoxify reactive metabolites of styrene formed *via* CYP2F2 metabolism. These reactive/toxic intermediates include styrene oxide and most probably the downstream metabolites of 4-VP. Detoxification of toxic species by epoxide hydrolase is 10-fold higher in the rat olfactory tissue as compared to the mouse while glutathione S-transferase activity is approximately 3-fold higher in the rat nasal tissue as compared to the mouse. It is important to consider that cytochrome P450-catalysed metabolic activation of styrene to styrene oxide in human nasal epithelial tissue appears to be negligible (see toxicokinetics section). Also, since uptake of styrene by nasal tissue is enhanced by the ability of the tissue to metabolize styrene, the lack of the primary step of styrene metabolism in human nasal tissue serves as a further reason for dismissing the relevance to humans of these rodent nasal lesions. Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which result in significant different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is likely that high levels will not be deposited in the olfactory area. Furthermore, human investigations have shown that exposure up to 77 ppm (8h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function, and in several human health surveys of workers repeatedly exposed to styrene up to approximately 700 ppm, no nasal lesions have been described (see RDT, human studies section). Hence, it can be concluded that rodent nasal epithelium damage induced by styrene is not of relevance in relation to the potential repeated dose effects of styrene in humans at relevant levels of exposure.

In relation to the lung, no effects were seen in rats exposed up to 1000 ppm, but in mice a NOAEC was not identified, with damage to the lung epithelium (hyperplasia in the terminal bronchioles, focal bronchiolar-alveolar hyperplasia, fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct) having been seen from 20 ppm, the lowest exposure concentration tested in a 2-year study. Work with the cytochrome P450 inhibitor 5-P-1-P has shown that metabolic activation of styrene to styrene oxide and other reactive metabolites (e.g the downstream metabolites of 4-vinylphenol) and the subsequent detoxification of styrene oxide are crucial elements of this toxic response. This is supported by the observation that it is the metabolically active Clara cells that are the initial focus of damage. These non-ciliated bronchiolar epithelial cells are mainly involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium (Komaromy and Tigyi, 1988). Early biochemical changes, sustained cell damage and regenerative cell proliferation were observed in lung Clara cells of mice exposed to 40 and 160 ppm styrene for up to 4 weeks. In this context it is important to consider that cytochrome P450-catalysed metabolic activation of styrene in human lung tissue appears to be minimal (see toxicokinetic section) even though the two main P450 isoforms involved in styrene metabolism, CYP2E1 and CYP2F1, have been detected; that the number of Clara cells in human lung is very low; and that their most important fucntions in human lung are shared by other cell types (e.g. Type II cells). Hence, it can be concluded that these lung tissue findings in mice reflect a toxic response that will not occur to any significant extent in humans at relevant levels of exposure.

In relation to the liver, exposures in the range 150-350 ppm have produced fatal hepatotoxicity in mice. Again, it appears that the metabolic activation of styrene to styrene oxide is a crucial stage in the hepatotoxicity process. In this context, it is evident that the toxicokinetic information available suggests that the mouse would be more susceptible than

humans to styrene induced hepatotoxicity. This is borne out by the observation of mouse fatalities at exposure levels to which humans have been regularly exposed in the past, with no reports of hepatotoxicity or death.

In relation to effects on the ear, clear evidence of ototoxicity (both functional and histological has been seen in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks' duration. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tend to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm.Comparative studies using rats and guinea pigs exposed to 1000 ppm for 5 days indicate an obvious species-difference, as similar findings were not observed in guinea pigs. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. The underlying toxicological mechanism has not been clearly elucidated. This effect should be regarded as of potential relevance to human health. The histological damage consists in the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irriversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organisation of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. On this basis, it is considered that the NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively are a more accurate starting point for risk characterisation than the NOAEC of 200 ppm for 13 weeks.In one single non-standard investigation, rather minor effects on the number of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks have been reported. However, it is considered that, in the absence of any associated conventional histopathology and given the lack of of information on what are the 'normal' levels of dopamine and glutathione in the retinal cells of rats, the rather minor morphometric and biochemical changes observed are unlikely to represent a toxic response.

Overall, the available inhalation repeated dose toxicity studies have identified ototoxicity as the most sensitive and relevant effect of styrene repeated inhalation exposure with NOAEC values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) for 4 weeks in sedentary/ordinary and active rats respectively.

Most of the available repeated oral exposure studies have been performed in rats and mice. Information from a carcinogenicity bioassay in the rat has shown no evidence of treatment related toxicity in animals administered 1000 mg/kg/day for two years; a marked increase in mortality was evident at 2000 mg/kg/day. In another 2-year study, styrene did not produce any clear evidence of toxicity when administered in drinking water at a dose of 21 mg/kg/day, the highest dose level tested. However it is noted that potential effects on the ear were not investigated in these studies. Ototoxicity, a clearly established effect of styrene in the rat, was seen in one study at 800 mg/kg/day (and perhaps, although less convincingly, at 400 mg/kg/day) for 2 weeks.

In mice, the most reliable information comes from a cancer bioassay, in which increased mortality and hepatic necrosis were observed at the highest dose of 300 mg/kg/day; a NOAEL of 150 mg/kg/day was identified from this study. The one significant observation from the remaining studies is that of toxicity towards the lung epithelium, adding further support to the concept that the lung toxicity of styrene in mice, following oral and inhalation exposure, results from local metabolism of styrene to styrene oxide and to other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol).

Overall, in relation to repeated oral exposure, the NOAEL of 150 mg/kg/day identified from a 2-year cancer bioassay in the mouse should also be considered. But in extrapolation to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat. No repeated dermal studies are available, although low systemic toxicity would be predicted in most conventional experimental species with the possible exception of some strains of the mouse.

4.1.2.6.2 Studies in humans

The effects of repeated styrene exposure in humans have been studied extensively but the value of many of the studies, for regulatory purposes, is limited by a lack of precision regarding the styrene exposures of experienced by affected individuals or the failure to compare the exposed subjects with a suitable control group. As styrene is known to have some potential to affect the CNS (producing mild narcotic symptoms) following single exposures, there has been particular emphasis on investigating its potential to produce other neurological and psychological (neurobehavioural) effects on long term exposure. For clarity of presentation this section of the review has been divided into sub-sections.

Mortality studies

Based on the data obtained by Wong (1990) and Wong *et al* (1994) (see section 4.1.2.8.3), from the investigation of cancer mortality in workers employed in the U.S. reinforced plastics and composites industry and exposed to styrene, the cohort has been further analysed with respect to mortality from non malignant diseases of the respiratory, genitourinary and nervous systems (Wong and Trent 1999). For all workers the incidences of deaths from all diseases of the nervous system (observed 14 deaths) and of genitourinary diseases (observed 13 deaths) were lower than that expected from U.S. national rates (24.7 nervous system deaths and 14.8 genitourinary deaths, respectively). Increased rates were observed with respect to non-malignant respiratory disease (97 deaths observed compared with 80.2 expected). Twenty three (23) of these deaths were due to pneumonia (expected 24.9), 23 were classified as being due to ‘bronchitis, emphysema or asthma’ (expected 18) and 51 were reported as being due to other non-malignant respiratory diseases (expected 36.3). Thirty six (36) of these latter deaths were reported as being due to “chronic airway obstruction, not otherwise specified” which included chronic non-specific lung disease, chronic obstructive lung disease and chronic pulmonary disease, not otherwise specified. Mortality analysis by duration of exposure and/or employment indicated that there was no pattern evident with respect to mortality incidences for any of the disease types investigated. Short term workers (<1 year exposure) had the highest mortality from non malignant respiratory disease (SMR 1.41), whereas there was no increased mortality from non malignant respiratory disease in those workers with >10 years exposure to styrene. Overall, in this study there was no evidence of a relationship between styrene exposure and any increase in worker mortality due to diseases of the nervous system, non-malignant respiratory, or other diseases.

Another study investigating mortality from non-malignant respiratory disease in workers with styrene exposure is available (Welp *et al,* 1996a). A cohort previously used by Kogevinas *et al* (1994) to investigate possible cancer risk (see section 4.1.2.8.2) was assessed, comprising 34560 men and 6128 women employed in 660 European factories manufacturing reinforced plastic products. Individuals unexposed to styrene or with unknown styrene exposure (n=5245) were excluded from the study. The remaining 35443 known styrene exposed individuals remained in the cohort for comparisons with national reference rates. The criteria for the selection of individuals into the cohort, the follow up period, individual loss rates and the determination of styrene exposure levels are reported in section 4.1.2.8.2.

Overall mortality from "all causes" amongst the exposed workers was lower than expected from national rates (2714 deaths, SMR 0.92). Mortality deficits were found for "all non-malignant respiratory diseases" (118 deaths, SMR 0.81), pneumonia (27 deaths, SMR 0.62) and bronchitis, emphysema & asthma (63 deaths, SMR 0.86). The only trend identified in connection with styrene exposure was that mortality from all non-malignant respiratory diseases showed an increase with increasing time since first exposure (<10 years SMR 0.58; 10-19 years SMR 0.89; >20 years SMR 0.92). However, all SMR values remained below 1 and there was no relationship with other indicators of styrene exposure. Mortality from pneumonia also increased with time since first exposure, and with average exposure to styrene, but not with cumulative exposure or duration of exposure. The authors reached the balanced conclusion that, in the absence of verification in other studies, the finding might represent a true casual effect of styrene but might also be a chance finding.

Using the same cohort, the same workers have investigated possible relationships between styrene exposure and mortality from non-malignant diseases of the genitourinary system, (Welp *et al*, 1996b). There was no elevation in the number of deaths from non-malignant diseases of the genitourinary system in exposed workers compared with national rates (SMR 0.94). There was a correlation of the mortality rate with increasing styrene exposure. However of the 20 worker deaths in this general category, 5 were from nephritis and nephrosis, 3 from renal failure, 10 from other diseases of the urinary system, 1 from a prostrate disorder and 1 from an ovarian/fallopian tube disorder. Such a varied pattern of diseases would not have a common etiology. Although the authors suggest that the potential association with styrene is worthy of further exploration, there was no convincing evidence of an effect of styrene exposure on diseases of the genitourinary system in this study.

Mortality from non-malignant disease has been investigated in a cohort of 36610 styrene exposed workers employed between 1964 and 1988 at 386 facilities in Denmark (Kolstad *et al*, 1995). A reference population of 14293 non exposed workers, in the same plants, was also investigated. No details of medical histories or smoking habits were reported. No information regarding potential exposures to other chemicals was reported. No data on individual exposures or job titles were available. Employees of a company with <50% of the workforce involved in work with reinforced plastics were classified as having "probable low styrene exposure", whereas employees of companies with <50% of the workforce working with reinforced plastics were classified as having "probable high styrene exposures". This is a very crude means of exposure discrimination. Duration of employment was determined from payment records. Overall mortality was slightly increased compared against Danish national rates, with a total of 3031 deaths, in the cohort (expected 2754.1 SMR 1.10, 95% CI 1.06-1.14). However, there were no notable elevations in mortality for any particular non-malignant diseases. For degenerative disorders of the nervous system and cardiomyopathy, workers in the "high exposure" group had a higher mortality rate than in the "low exposure" group. However, the differences were not statistically significant. Overall, the study presents no convincing evidence of an association between styrene exposure and elevated mortality from non-malignant disease.

In addition to the analysis of death due to cancer, Ruder *et al* 2004 (see also Section 4.1.2.7.4, Carcinogenicity) investigated other causes of mortality. There were no statistically significant or consistent increases in SMRs for non-cancer deaths in this follow-on study.

A case-cohort study examined the relative risks of death due to ischaemic heart disease (IHD) from two styrene-butadiene polymer-manufacturing plants in USA (Matanoski and Tao, 2003). The target population was male workers (n=6587) employed between the start of the industry in 1943 until 1982. Females were excluded because of a lack of information about job history. There were 498 cases where IHD was the underlying cause of death or diagnosed as being contributory. Non-cases were a 15% random sample of the 6587 workers (n=997, including 71 who eventually died of IHD – these were included as referents in the study up to the point of death). Exposure information on styrene and butadiene was either from measurements or estimation based on job history. Smoking status (an obvious potential confounding factor) was determined from a sample of 424 questionnaires that had been returned from cases and controls. However, as smoking was not correlated with styrene exposure it was concluded not to be a confounding factor and was not corrected for in the analysis. One weakness of the study is that the respondents to the smoking survey were a relatively small fraction of the total workforce which may not be truly representative. A proportional hazards model was used to analyse results, and age was used as a matched variable. The model also corrected for race and considered the number of cases and non-cases amongst current workers, those who had left 10 or more years previously and those who had left less than 10 years previously. Workers were grouped into those who had been 'ever-employed', employed for 2 or more years, and employed for 5 or more years – these groups were inclusive of each other.

Amongst active workers there was a slight increase in risk of death due to acute IHD once butadiene exposure had been taken into account suggesting an association of cumulative exposure to styrene with acute IHD (a relative hazard, RH = 1.04, 95% CI 1.00-1.09 amongst those employed for 2 or more years and RH = 1.04, 95% CI 1.00-1.08 amongst those employed for 5 or more years). Curiously, and in contrast to this, a more pronounced and apparently dose-related effect was noted when the intensity of styrene exposure was estimated by job category. If the exposure modelling was accurate both methods should result in similar risk estimates; the inconsistency raises some doubts about the association of styrene exposure with IHD. Overall, the authors describe the apparent increase in risk of IHD as of "borderline significance". Amongst former workers there was no association between butadiene or styrene exposure and acute or chronic IHD.

In a further analysis of information obtained from the cohort mortality studies in the styrene-butadiene rubber (SBR) industry Delzell (2004, unpublished) also reports on the risk of acute and chronic ischaemic heart disease (IHD). From a cohort of 16759 men who had been employed for at least one year at any of 6 SBR plants previously described by this author and her team, 14006 were identified as potentially exposed to styrene. Between 1944 and the end of 1998, 5703 men died; of these, the cause of death was ascribed to IHD in 1684 cases (1021 with acute IHD and 663 with chronic IHD). Of these 1684 cases 1412 had been exposed to styrene. SMRs for IHD were calculated against the general population for the locality in which the factory was based (at US State or Canadian Province level) and were controlled for age, race and time period of death. The median number of years worked was 11.8, and median intensity of styrene exposure was 12.7 ppm-years (overall median styrene concentration 1.6 ppm).

From the 1412 deaths due to acute and chronic IHD amongst the styrene-exposed workers an overall SMR of 0.9 (95% CI = 85-95) was calculated; there were no significant differences according to race, age, locality, years since hire. For employees that had ceased working with styrene, there was marginal increase in SMR for IHD, 1.29 (95% CI = 0.93-1.75) but only for the first year since leaving styrene-related work. For subsequent years there was no increase in SMR for IHD. Also, there was no consistent trend with increasing styrene

exposure (as ppm-years or overall intensity). During that first year after styrene-related work, increased mortality was also observed for other major causes of death. Thus, the author suggests that there is a tendency for employees to stop work as they experience a serious, life-threatening illness.

Overall, therefore, the marginally increased SMR for IHD reported by Matanoski and Tao (2003) should not be considered to be styrene-related as no evidence emerges from a much larger study (Delzell, 2004) for a concern over death due to acute or chronic IHD associated with styrene exposure.

Summary of mortality studies (non-malignant disease)

In the few studies that have reported on patterns of mortality from non-malignant disease in occupational groups exposed to styrene the authors of these studies have signalled findings that have been proposed to be worthy of further exploration. However, a critical appraisal of the studies, taken together, suggests that they present no convincing evidence that styrene exposure has enhanced the incidence of mortality from any particular disease.

Worker health surveys

The results of a health survey of 488 workers at a factory in the USA where styrene was manufactured and polymerised have been reported (Lorimer *et al*, 1976; Lilis *et al*, 1978; Lorimer *et al,* 1978). The average age of the workers was about 45 years, and their duration of exposure varied from 0.1 year to over 20 years. These workers were divided into two subgroups: 288 workers in jobs classified as involving appreciable exposure to styrene and 200 men in low exposure or unexposed jobs. The latter included 41 workers who had been transferred to other areas in the site, or who had retired. No information was given as to the relative ages of the two subgroups, or their comparability with regard to other possible confounding or matching factors. Styrene exposure levels and duration of exposure for each worker were obtained from occupational histories and spot air sampling. Workers in the low exposure group (n=200) were reported to be exposed to <1 ppm as a TWA (duration not stated), whereas those in the high exposure (n=288) group were reportedly exposed to 5-20 ppm as a TWA. Group classification was confirmed from the results of mandelic acid and phenylglyoxylic acid analysis in urine samples. It was also stated that men from the “high” exposure group were sometimes exposed to levels of “hundreds of ppm”.

Each person was subjected to clinical examination, which included assessment of symptoms, haematology, liver function tests, lung function tests (spirometry), chest X-ray and ophthalmic examination. Nerve conduction velocity was also measured (see section on nerve conduction studies). No control groups were used except for the liver function tests. Symptoms described as “pre-narcotic” (lightheadedness, drunkenness, dizziness, headaches, nausea, loss of balance and un-coordination) were self reported by individuals in both exposed subgroups, the incidence being 19% in those exposed to the higher styrene levels as compared to 10% of the other group. This increase was statistically significant. Irritant effects on the eyes and nasal mucosa were noted to a similar extent in both groups. Evidence of lower respiratory tract irritation, consisting of episodes of wheezing or tightness in the chest, was reported in 19% of the former and 7% of the latter group; recurrent symptoms were noted in 12% and 5% respectively. In contrast, chronic bronchitis was less prevalent in the men exposed to the higher styrene levels (2% as compared to 8% in the lower exposure group). Lung function tests on the non smokers in both groups also indicated less impairment in those exposed to the higher styrene levels, with some evidence of airways’ obstruction (defined as $FEV_1/FEV<75\%$) in 25% of the high exposure group as compared to 36% of the low group. No evidence of any radiological changes in the lungs was noted in either group. The liver function tests consisted of measurement of serum

bilirubin, alkaline phosphatase, alanine aminotransferase and gamma-glutamyl transferase. The results from each sub group of styrene exposed workers were compared to those obtained from 993 men (not hospital patients) tested at the same laboratory; the average age of the latter group was about 42 years. The only significant difference noted was in the distribution of workers with 'abnormal' gamma-glutamyl transferase levels (defined as above the 96th percentile of the control group) after exclusion of subjects with elevated levels of alcohol consumption. The overall proportion in the exposed workers was 5% but seven per cent of the subgroup or workers exposed to the higher styrene levels had elevated levels as compared to 3% in the workers exposed to the lower styrene levels. The increased enzyme levels were also related to duration of exposure but no attempt was made to control for the increasing age of the exposed workers. No consistent pattern was seen for any other liver function tests. No significant differences were noted regarding the haematological parameters of the two groups. Ophthalmic examination revealed no evidence of optic neuritis, or any abnormality of the optic fundus in any of the workers examined.

Overall, the only effects noted in this study that appeared to be related to exposure to styrene were an increased incidence of pre-narcotic symptoms and also some symptoms of irritant effects on the lower respiratory tract, although limited lung function tests did not reveal any impairment. The small increase in the percentage of unusually high results for serum gamma-glutamyl transferase levels in those with high styrene exposures is not considered to be significant, particularly as no other markers of liver dysfunction were affected.

Styrene and polystyrene manufacture workers were compared with reference workers in a morbidity study by Theiss and Friedheim (1978). Eighty four workers employed in a styrene plant (exposure duration ranged from 1 to 36 years), and 93 workers employed in a polystyrene plant (exposure duration ranged from 1 to 38 years) were investigated. A reference group of 62 unexposed workers of similar "living" conditions was used. No further details of any matching are reported. All workers were questioned regarding their occupational and medical histories, and underwent physical examinations (height, weight, blood pressure, pulse rate, vital capacity, electrocardiograms and radiological examination of the thoracic organs) and provided blood and urine samples for laboratory testing (blood counts, thrombocyte counts, SGOT, SGPT, gamma-GT, lactate dehydrogenase, alkaline phosphatase, bilirubin, albumin, thymol test, urea and creatinine levels). Worker absenteeism and accident rates were also assessed. Atmospheric concentrations of styrene were measured at various parts of the exposure area, at the time of the investigation. In the styrene plant no exposures exceeded 10 ppm (further details not stated) and most were less than 1 ppm. In the polystyrene plant generally exposures did not exceed 8 ppm and most were less than 1 ppm, with one or two high exceptions. These were no increases in the occurrence of abnormalities in relation to any of the above parameters in the styrene exposed workers, relative to the control group.

Pulmonary function (vital capacity or forced expiratory volume) and liver function (serum ASAT, ALAT, ALP) were assessed in a small group of 17 GRP workers and compared with a group of age matched referents (Axelson and Gustavson, 1978). Eight hour TWA levels of 100-200 ppm styrene were measured in the breathing zone of the workers. Peaks of >1000 ppm were measured in the breathing zones of sprayers, who were said to be able to withstand the irritant effects of styrene vapour without the use of PPE. There were no significant differences between the two groups in any of the parameters assessed.

The results of a health survey of 98 men exposed to styrene at 24 different factories manufacturing polyester plastic products in Finland have been reported (Seppalainen and Harkonen, 1974 and Seppalainen and Harkonen, 1976). Urinary mandelic acid levels were monitored in each worker at the end of the work shift, once a week (on different days) for five weeks. The mean urinary mandelic acid concentration in the exposed workers was 808 mg/l

with the range observed being 7-4715 mg/l. Studies by the same group of workers indicated that this average madelic acid level was equivalent to an 8 hour TWA styrene exposure of the order of 35 ppm but exposures must obviously have been considerably higher in some workers, given the range of mandelic acid concentrations reported. Each worker was examined physically, and blood samples were taken for haematology and clinical chemistry. Urine samples were analysed for glucose and albumin, and in addition, were subjected to cytological investigation to determine whether any atypical epithelial cells were present. Lung function tests were also performed. An assessment of symptoms of toxicity experienced by the workers was also made using a questionnaire and the results compared to those obtained with a similarly sized control group; this consisted of local men, of comparable age, who were employed as postal workers or electricity workers, and were not exposed to styrene.

No significant difference was noted between the exposed and control workers, with respect to physical examination, haematology, clinical chemistry, urinalysis and lung function tests. Symptoms of fatigue and difficulty in concentrating were reported in a significantly greater proportion of the styrene workers as compared to the controls; 41% of the exposed workers felt excessively tired at the end of the work shift, and 24% stated that they frequently forgot things, as compared to 8% for both symptoms in the control group. Complaints of headaches and feelings of dizziness were also more frequent in the exposed group, as were irritant effects (itching and redness of the eyes). However, there was no correlation between any of the symptoms and urinary mandelic acid levels. One possible explanation is that the effects were due to short term peak exposures to styrene rather than average exposure levels. Overall, the effects noted in this study were an increased incidence of subjective symptoms of tiredness, memory loss, mild central nervous system effects and irritancy. The tiredness might possibly have been related to physical activity; the report does not include a comparison of physical workload in control and exposed workers. Other symptoms might well have been caused by styrene, but it is not possible to associate them with the precise exposure conditions responsible.

Medical examination of a group of 33 workers from 3 Swedish GRP factories revealed signs of eye and throat irritation amongst the workers (Rosen *et al*, 1978). Although some data is given in the report on mean atmospheric styrene levels, it is impossible to relate such data to the specific cases of eye or throat irritation.

A health survey has been carried out on a small group of workers exposed to styrene at three separate Dutch factories involved in the processing of reinforced plastic, mainly in boat making (Zielhuis *et al,* 1963). Average exposure over the work shift was estimated to be in the range of 24-94 ppm, but it is considered that the men would have been exposed to mean concentrations in the range 106-209 ppm while working in closed or partially closed boats; peak concentrations during such periods were estimated to be 235-705 ppm. The numbers involved were small, consisting of 5, 16 and 6 men from the separate factories. In addition a further group of 28 men exposed to styrene during processing of reinforced plastics was examined. These men were exposed to lower styrene levels (average airborne concentrations estimated to be about 7 ppm but with maximum concentrations in the range 14-74 ppm). The health survey results were compared with those obtained in a control group consisting of 5, 21 and 5 workers from each of the 3 factories; these controls were involved in jobs that were unlikely to result in any exposure to styrene. All of the workers in the study had been employed for less than 5 years but no further details were given regarding their age, or the comparability of the exposed and control groups.

Each worker was subjected to a physical examination, blood tests (including haematology, blood bilirubin and protein determinations) and urine analysis. In addition symptoms were assessed using a questionnaire. The only differences noted between the exposed and control workers were in the results of the questionnaire. These indicated an increased incidence of irritation of the eyes and nasal mucosa, and also increased drowsiness,

anorexia and dizziness reported by the workers exposed to styrene. The increase was most marked in those exposed to the higher levels of styrene, but some symptoms were also reported in those exposed to the lower levels (maxima estimated to be 14-74 ppm). In all cases the symptoms only occurred during the work shift, and quickly disappeared after leaving the work area. No increase in absenteeism was noted in any of the exposed groups.

Results of medical examinations of a group of 50 workers exposed to styrene at two factories in the USA have been reported (Zielhuis *et al*, 1961). No data were available on the atmospheric styrene levels present. In addition to styrene these workers were known to be exposed to epoxy resins and volatile polyamine hardeners (mainly triethylenetetramine) and the contact dermatitis reported in 9 of the men was thought to be due to these other chemicals. The medical examination included haematology, blood biochemistry, liver function tests, and urinalysis. The only adverse effects noted were in a few subjects who reported subjective symptoms of drowsiness and “slight tremor”. This study contains no information on the styrene levels present and is very limited in its scope and value.

A group of 68 workers in the glass reinforced plastics (GRP) the Netherlands was asked 13 questions about health complaints at work (Geuskens *et al,* 1992). A control group of 111 workers exposed to organic solvents and paint dust at a paint manufacturer had also answered the same questions in another unpublished study. Factors such as smoking and drinking, work hygiene and conditions were said not to have been different between the two groups. Mean styrene exposure levels were 23-51 ppm, 8 hour TWA (geometric mean). The number of subjective symptoms reported (irritation e.g. headache, burning eyes, itching skin and pre-narcosis e.g. nausea, dizziness) was statistically significantly increased in the styrene exposed group, although the actual differences in questionnaire scores between the groups was small. It was felt by the workers that styrene and methylene chloride were frequently the cause of the complaints. Given the confounding presence of methylene chloride the small increase in the symptoms experienced by the GRP workers cannot be reliably attributed to styrene.

The health of workers at two Polish GRP factories has been investigated (Chmielewski *et al,* 1973). In the initial study 48 workers from one factory and 53 from the second were subjected to medical examination, including limited haematology and blood biochemistry (serum protein and lipid levels and liver function tests). Only very brief details were given regarding the atmospheric styrene levels; these were stated to be 2-6 times the stated MAC value, which would therefore equate to 25-70 ppm. No details were given on the maximum styrene levels present. There was no control group. Symptoms were reported by 26% of these workers, including excitability, perspiration, hypoaesthesia, whitening of the fingers and trembling. No adverse effects were noted on any of the blood parameters measured, nor on the liver function tests. In the absence of a control group, no reliable conclusion can be drawn from this study.

In a separate study glucose tolerance tests were carried out on 61 of these workers. These were divided into two groups on the basis of their length of exposure to styrene. These subgroups consisted of 40 workers with average exposure of about one year and 21 with average exposure of ten years or more. The results were compared with those obtained in a control group of 18 persons; no further details were given regarding the composition of this group, or the age of the subjects in any of the groups. The pattern of results obtained were unexpected. Markedly higher glucose tolerance, relative to controls, was noted in those workers with the shorter exposure to styrene. However, an appreciably lesser elevation, relative to controls, was noted in those workers with an average of ten years exposure to styrene. This study report is deficient in important details, particularly regarding matching of the groups involved. There is also no plausible explanation offered to the potential underlying mechanism and the pattern of the results. Overall, this study is considered to be unreliable.

A small group of GRP workers at 4 Czechoslovakian factories underwent medical tests (Huzl *et al*, 1967). Average atmospheric styrene levels were believed to be about 47 – 94 ppm. The numbers of workers involved at the 3 factories were 13 (including 3 women), 7 and 20 (including 7 women) respectively. At the fourth factory the laminates were handled mechanically, and ventilation was much better; average airborne styrene levels were below 6 ppm; fourteen workers (including 7 women) were investigated from this plant. Each worker was subjected to medical examination including haematology tests, test for liver function, neurological examination and electroencephalography. No control group was used. The results for the styrene exposed workers included headaches, exhaustion or drowsiness and dyspeptic disturbances. The highest incidence was stated to be in the group from the third factory, comprising mainly women. No abnormalities were noted in haematology. In relation to the assessment of liver function, “elevated” serum bilirubin levels, presumably above a reference value for the investigators’ laboratory, were noted in 17% of the exposed workers and a “prolonged” Weltman reaction occurred in 48%. These effects were stated to occur more frequently in the workers exposed to the higher styrene levels. However, there were no effects on serum aspartate or alanine aminotransferase levels and in view of this, and the absence of a control group, these results do not present convincing evidence of liver dysfunction. In the absence of a control group, this study does not present reliable information on the effects of styrene exposure. A limited health survey of workers exposed to styrene at a Russian factory has been reported (Kats, 1962). No information was given on the atmospheric styrene levels. A total of 526 workers (250 men, 276 women) was studied but not all tests were performed on all subjects and it is unclear on what basis the various subgroups involved were selected; in some cases they appear to comprise those workers who attended the factory clinic for particular treatments. The study does not appear to have any rational overall strategy. Symptoms reported included headaches, nausea, heartburn, and heavy sensation or pain in the right subcostal region. Physical examination was said to have revealed liver enlargement in 159 workers. Biochemical studies were performed on various subgroups, the size and nature of which are unclear. Blood bilirubin levels were slightly higher than expected values in 19 subjects. In 63 selected subjects presupposed to have “styrene-induced hepatic pathology” there was a reduction in blood albumin and a rise in beta-globulin and, to a lesser extent, gamma-globulin as compared to the results from blood samples taken from “healthy” volunteers. Haematology, presumably on this subgroup, revealed a tendency forwards leucopenia (in 31% of the group) and reticulocytosis (in 59%). the quality of the methodology and reporting is too poor to permit any useful conclusions to be drawn. There is also no information given on possible confounding factors, such as the alcohol consumption of the subjects or the very probable co-exposure to butadiene and other chemicals.

Summary of worker health surveys

In the available worker health survey studies, consistent evidence of an increase in the self-reporting of symptoms of eye and nasal irritation and CNS disturbance (drowsiness, headache, light-headedness) comes through. Unfortunately, the quality of the exposure data means that it is not possible to relate these effects to reliable levels of styrene exposure, particularly as it is possible that these effects are related more to short-term peaks in exposure, rather than to workshift averages. No reliable evidence for any other effects of styrene is indicated by these studies.

Studies focussed on haematological, hormonal or renal endpoints

Workers (221) exposed to styrene in the GRP industry were examined in a haematological survey and compared with a group of 104 controls (Stengel *et al,* 1990). Age, sex and smoking habits were similar in each group. The majority of major haematological parameters

did not differ between styrene-exposed and all control workers. There were statistically significantly lower mean values of neutrophils and mean corpuscular haemoglobin concentration (MCHC), and statistically significantly higher mean values for monocytes and mean corpuscular volume, in the exposed group relative to controls, although these differences were very small. There was a dose-response trend in MCHC with values decreasing as urinary styrene metabolite concentration increased. Regression models were applied to the data. The above results remained significant for an association with styrene after adjustment for age, sex, tobacco and alcohol consumption and place of residence, with the exception of neutrophil changes, which appear to be accounted for by smoking habit. Nevertheless, the differences seen between the styrene-exposed and control workers were minor, and of no clinical significance. They have not been reproduced in other studies involving haematological investigations.

In another study, more detailed haematological investigations were carried out on 122 men from the two Czechoslovakian factories in the worker health survey by Huzl *et al,* described above (Chmielewski and Renke, 1976). The workers were divided into two groups on the basis of their length of exposure of styrene. These consisted of 101 workers with an average exposure of about one year and 21 with an average exposure of about ten years. Blood cell counts were in the normal range for all workers. A statistically significant but small difference in mean platelet levels was evident, being lower in those with longer exposures. The observation of a slight reduction in mean platelet levels in these workers prompted an investigation into the coagulation/fibrinolysis system in the group of workers with longer exposure to styrene. The results were compared to those obtained in a control group of 20 persons, but no further details were given regarding this group. No difference was noted in bleeding time. Coagulation time was statistically significantly longer in the styrene-exposed workers, compared with controls. Other differences noted included a lower prothrombin ratio and a shorter time for blood euglobulin fibrinolysis (both statistically significant) in the styrene-exposed group compared with the controls. However, although mean platelet numbers were lower in the longer exposed workers, their adhesiveness was statistically significantly higher than in the controls. Overall, the results appear to reflect small differences on blood clotting parameters that would operate in opposing biological directions. The findings also stand in isolation. Hence, it is considered that they should not be regarded as reliable findings.

A haematology survey was carried out at a styrene-butadiene manufacturing plant (Checkoway and Williams, 1982). A group of 154 workers gave blood samples. The mean 8-hour TWA concentrations were highest (14 ppm styrene and 20 ppm butadiene) in one area of the plant known as the tank farm. In tank farm workers, red blood cell count was slightly lower than in other workers, but the difference was clinically insignificant. The contribution from styrene exposure was analysed by multiple regression analysis; little effect of styrene was found using this method. Overall, in this limited study there was no evidence of an effect of styrene on haematological parameters.

Georgieva *et al* (1998), have reported that in a cohort of 110 women occupationally exposed to benzene, toluene, xylene and styrene, mean RBC and haemoglobin levels were significantly lower than in a control group of 45 unexposed women from the same plant. However, given the mixed exposures, no conclusions regarding a causal relationship with styrene can be drawn from this study.

The status of the immune system in 71 styrene-exposed workers and in 65 unexposed control subjects has been investigated (Bergamaschi *et al,* 1995). Workers and controls were selected using the same criteria and were matched in terms of sex, age, and confounding variables. All individuals were questioned with respect to their age, smoking and drinking habits, medical and work histories. Exclusion criteria included: current or recent infection or fever; autoimmune disorders; haematological disorders; allergies; and the

frequent use of drugs which could interfere with the immune system. Air and biological monitoring were used to characterize styrene exposures. Immune system status was determined by flow cytometric analysis of the phenotypic conformation of the peripheral blood lymphocytes (PBLs). The cohort consisted of 39 men and 32 women, average age 32 years, 33 of whom were smokers. The mean duration of styrene exposure for the cohort was 7 years. The control population consisted of 34 men and 31 women, average age 34 years, of whom 25 were smokers. An additional group of 14 styrene-exposed workers (average age 39 years) was recruited for functional studies on natural killer cell (NK) activity. Styrene exposures were found to range from 10 to 50 ppm (8-hour TWA). PBL analysis revealed similar mean lymphocyte counts in workers and the control populations. The proportions of some T-lymphocyte subsets were lower (by 11-25%) in exposed groups compared with the control population. Conversely, the proportions of some B-lymphocyte subsets were higher (by 19-37%) in workers compared with controls. The proportions of immunoglobulin subclasses were comparable in workers and controls. Expression of some NK-related phenotypes was increased by about 30% in workers compared with controls. There is no established framework within which to interpret the toxicological significance of the results obtained. In view of this, it is suggested that the results be disregarded in the context of constructing the toxicological profile of styrene.

A group of 32 GRP workers and a control group of 19 carpentry workers, similar in age and sex, were examined in a study of lymphocytes (Mutti *et al,* 1992). Several exclusion criteria were applied to the groups. The styrene-exposed group of 22 males and 10 females was split into subgroups exposed to levels of styrene either above or below 50 ppm, 8-hour TWA. The subgroups were similar in age and sex. Peripheral blood was sampled. In the >50 ppm group helper T-lymphocytes were significantly lower in number and suppressor T-lymphocytes and natural killer cells were higher, relative to the control group. These differences were not apparent for the >50 ppm group. However, the numbers used in control and exposed groups were very small and these parameters show significant variation with in the general population. Without confirmation of the result and a hypothesis to explain the underlying process involved, these results cannot be considered to be a reliable reflection of styrene toxicity.

Hormone levels were studied in a group of 30 female GRP workers and 30 age-matched controls who were factory workers from the same area (Mutti *et al,* 1984). There were no clinical histories of endocrine or neurological disease in either group. Only subjects in the proliferative phase of the menstrual cycle were included. Urinary metabolite levels were used to estimate the average airborne exposure to styrene, which was given as 130 ppm. In the styrene-exposed group serum prolactin and human growth hormone levels were statistically significantly higher, by about two fold, compared with control group values. No significant differences were observed between the groups in the levels of thyroid stimulating hormone and gonadotrophins. Again, in the absence of confirmation of the results and explanation of the findings and their biological significance, these differences cannot be considered to be a reliable reflection of styrene toxicity.

A study of renal function and integrity in styrene-exposed workers is available (Verplanke and Herber, 1998). For a four-day period, urine samples were collected overnight and at the end of shift from two groups of 5 styrene-exposed workers (employed for a mean of 12.6 years) and from a total of 15 non-exposed workers. Styrene exposure was estimated by extrapolation from the sum of the concentration of the urinary metabolites mandelic acid and phenylglyoxylic acid. Effects on renal function and integrity were assessed by measurement of the urinary parameters alanine aminopeptidase (AAP), β-galactosidase (βGAL), N-acetyl-β-D-glucosaminidase (NAG), retinal-binding protein (RBP) and albumin (ALB). Exposures to styrene were estimated to range between 5 and 93 ppm (8-hour TWA). The only differences between the results in exposed and non-exposed groups were for RBP in overnight urine (RBP in workers was 69.2 compared with 40.8 in controls) and ALB in the end-of-shift

samples (1.85 compared with 1.28 in controls). These differences were not statistically significant at 5% level and the pattern of differences is inconsistent with a genuine effect on the kidney. In conclusion, this study presents no evidence of an effect of styrene exposure of kidney function.

Summary of studies focussed on haematological, immunological, hormonal or renal endpoints

From the studies available there is no convincing evidence of a clear, interpretable and toxicologically significant effect of styrene having occurred in exposed workers, in relation to these biological areas.

Neurological studies in workers occupationally exposed to styrene

There is substantial literature exploring the potential effects of styrene on the nervous system (including hearing function and colour vision) in humans. This section is therefore divided into a number of discrete sub-sections dealing with different foci of investigation.

Mortality studies

Mortality from degenerative disorders of the nervous system has been investigated in workers employed in the Danish reinforced plastics industry and exposed to styrene and in a group of workers without exposure to styrene (Kolstad *et al*., 1995). This study is reported in the previous section. In relation to diseases of the nervous system, there were 24 cases with an underlying cause of death attributed to degenerative disorders of the nervous system and 60 where there was an underlying or contributing cause. Of these 60, 7 were multiple sclerosis, 24 parkinsonism, 24 other diseases of the brain and 5 motor neuron diseases. In comparison with national rates the SMR for degenerative disorders of the nervous system for those in styrene-exposed industries was 0.94 (95% CI 0.65–1.50) and for the comparison group 0.82 (95% CI 0.30-1.69). In the Poisson regression those with high exposure probability had a MRR of 1.8 (95% CI 0.8-4.3) compared with the unexposed comparison group. The MRR was higher in those with longer duration of employment. Overall, degenerative disorders of the nervous system appeared to occur more often amongst workers with a high probability of exposure to styrene than in comparable non-exposed workers. However, the relatively large confidence intervals reported which do not rule out a chance finding, the rather crude estimate of exposure by the division of companies into those with low and high probability of exposure, the relatively small numbers of deaths, the very short follow-up with only 9% of deaths within the cohort and the lack of information on important workplace or lifestyle (e.g. alcohol intake) confounders make the findings of this study difficult to interpret.

In an international cohort study described in detail in the carcinogenicity section, mortality from nervous system diseases, mental disorders and suicide was examined in relation to styrene exposure (Welp *et al,* 1996c). The cohort comprised 41,167 subjects employed in GRP plants during 1945-1991 in several European countries. Those unexposed to styrene and those with incomplete information were excluded leaving 32,802 subjects. Following collection of styrene exposure measurements and job descriptions, indicators of exposure were derived and subjects were divided into different exposure categories. Standardised mortality ratios were calculated using appropriate country-specific rates.

Mortality from all causes in the total cohort was lower than expected (2196 deaths, SMR 0.92, 95% CI 0.88-0.95). Mortality from mental disorders (21 deaths, SMR 1.01, 95% CI 0.62-1.54) and from peripheral and central nervous system diseases (30 deaths, SMR 0.76,

95% CI 0.51-1.09) was no higher than expected. Looking at the narrower categories, there were no deaths from peripheral nervous system disease. An excess of suicides and deaths from violence caused a significantly raised mortality in the accident, suicide, violence category (365 deaths, SMR 1.13, 95% CI 1.02-1.25). There was some indication that factors other than styrene accounted for this increase as the highest risk was in the short-term workers. Poisson regression was used to assess mortality with respect to exposure indicators. The mortality from CNS diseases increased with increasing average exposure to styrene (p value for trend 0.37) and cumulative exposure (p=0.32). Subjects with an average exposure higher than 120 ppm were at an almost 2-fold higher risk compared with workers whose average exposure was <60 ppm. Mortality from degenerative diseases of the CNS did not increase consistently with increasing exposure to styrene (although mortality tended to be higher in workers with high cumulative or long duration of exposure). Deaths from epilepsy were approximately the number expected but did increase with exposure indicators. Since mortality from cerebrovascular diseases (stroke) was not associated with exposure it is unlikely that the positive trend for CNS mortality is due to accidental transfer of diagnosis between these groups. However, the cause of death included in the CNS disease mortality statistics were diverse and unrelated which would indicate a decreased likelihood of a causal link with styrene. In this study, an association was found between increasing extent of styrene exposure and mortality from CNS disease. However, the relatively small numbers of deaths from any given cause within this category (e.g. meningitis, anterior horn disease), the very short follow-up with only 7% of deaths within the cohort and the lack of information on important workplace or lifestyle confounders make the findings of this study difficult to interpret.

Mortality from non-malignant diseases of the nervous system has been investigated in workers employed in the U.S. reinforced plastics and composites industry and exposed to styrene (Wong and Trent, 1999). This study is reported in the previous section. In relation to nervous system diseases, for all workers the incidence of deaths from all diseases of the nervous system (observed 14 deaths) was lower than that expected from U.S. national rates (24.7 nervous system deaths). Mortality analysis by duration of exposure and/or employment indicated that there was no pattern evident with respect to mortality from nervous system diseases.

Electroencephalographic (EEG) studies

Although there are several studies investigating the effect of styrene on EEG patterns, a major problem is the lack of a clear, validated and generally accepted interpretational framework for assessing the significance of the results. As electroencephalogram (EEG) can vary with arousal, it is very important to control for the state of arousal of the subject. It is not clear that this was done in all cases. CNS-depressant chemicals or pharmacological depressant agents have the potential to produce diffuse, non-specific changes in EEG patterns. Lesions such as epileptiform foci could also be detected by EEG techniques.

All workers in the Finnish study reported above underwent electroencephalography (Seppalainen and Harkonen, 1976; 1977; Harkonen *et al,* 1978a and 1978b). The findings were interpreted according to clinical criteria described by Kiloh and Osselton (1966). EEG abnormalities mostly comprised excessive diffuse theta activity in 8 workers and localised slow waves in 14 workers. Nine of the latter involved the posterior regions. In addition, two subjects showed rare paroxysmal abnormalities consisting of bilateral spike and wave discharges. No control group was used in these studies, but the incidence of EEG abnormalities (24%) was significantly higher than that expected in the normal population. Previous studies at the same Institute and from the literature indicate that about 10% of a normal population would be expected to show abnormalities (Harkonen *et al,* 1978a and 1978b). Furthermore the incidence of abnormalities in the styrene-exposed population correlated with urinary mandelic acid levels. The incidence in workers with such levels below

700 mg/1 (n=38), and in the range 700-1999 mg/1 (n=43) and 2000-4779 mg/1 (n=15) was 10.5%, 32.6% and 33.3% respectively. From earlier data obtained at the same Institute these mandelic acid concentrations were claimed to be equivalent to average 8-hour TWA exposures of the order of 30 ppm, 30-100 ppm and greater than 100 ppm. In a later study the relationship between urinary mandelic acid levels and EEG abnormalities in this group of workers was investigated in greater detail, the authors having the objective of identifying a threshold for any effects of styrene on EEG pattern. The number of abnormal EEGs among the subjects with exposure less than 700 mg/1 was not above that found in the general population. An increase in EEG abnormalities was noted in the workers with urinary mandelic acid concentrations in the range 700-799 and 800-119 mg/1, values of 30% and 41% being noted in these two groups respectively. The incidence in those with mandelic acid concentrations above 1200 mg/1 was 29%. Overall the two studies showed an increased incidence of abnormalities in EEG in styrene-exposed workers.

In Finland, 100 male workers exposed to styrene were clinically assessed and submitted to thorough quantitative EEG. Any workers with a history of neurological disease, brain injury or serious relevant disorder were excluded (Matikainen *et al,* 1993). The number of exposed workers in total was not reported. Airborne styrene concentrations (measured during an 8-hour working day) had a “mean” of 29.5 ppm and reached a maximum of 183 ppm. The relationship of these values to personal exposures is unclear. The average duration of exposure was 12.7 years. Years at work, job specification, exposure concentration and urinary metabolite level were used to assign exposure categories. The low, medium and high exposure groups comprised 44, 35 and 30 workers respectively. There was no control group. Various symptoms were reported by each group and some, e.g. poor memory, were significantly more numerous in the high exposure category (39% of subjects vs 8% in the low exposure category). Observations of “slight abnormalities” were made in some workers in the clinical neurological examination but these did not correlate with the styrene exposure index. An important feature of the electroencephalography investigations was that the authors recognised the potential for artefactual abnormalities due to variations in arousal and took care to avoid recording during periods when the subjects might be drowsy. A technician maintained a conversation with subjects during the recordings to ensure that they remained alert. (This differs from typical EEG practice, where the recordings are taken with the subject relaxed). On conventional visual inspection of the EEGs by a clinical neurophysiologist, there was no increase in the number of abnormal EEGs. The EEG patterns were examined by a quantitative method not normally used for clinical assessment, which compared the amplitude, frequency distribution, asymmetry and coherence of the subjects tracings with normative data. The only difference between the groups which approached statistical significance was an increase in absolute EEG power (amplitude) in alpha and beta bards in frontotemporal brain regions in the high exposure group. The authors stated that this finding would not be regarded as medically abnormal. It is therefore concluded that in this study there was no evidence of any dose-related EEG abnormality of clinical significance.

The results from a study to investigate the potential for any neurophysiological effects of styrene in a group of 33 male workers from 3 Swedish GRP or polystyrene factories have been reported (Rosen *et al,* 1978). Mean styrene exposures derived from personal sampling in 1974 at two of these plants, were 125 and 47 ppm respectively. Apparently levels had been higher previously. At the third plant, levels were stated to have been not above 5 ppm. The values were time-weighted averages but the specific time period was not given. The mean age of the men from each factory was 44, 43 and 44. The results obtained were compared to those obtained from a small group of six employees at a local hospital, whose mean age (36) was slightly lower than the styrene-exposed workers. Each individual was subjected to neurological examination, including determination of motor nerve conduction velocity (fast fibres only) using several peripheral nerves and assessment of sensory neuropathy by determination of sensory action potentials using the median and ulnar nerves. In addition EEG tracings were obtained.

Results from a questionnaire indicated a higher incidence (46-85%) of subjective symptoms of tiredness, reduction in short-term memory and giddiness amongst the group exposed to the highest styrene levels compared with controls (0-33%). Medical examination revealed signs of eye and throat irritation amongst the workers, but no signs of polyneuropathy. There were no differences in motor nerve conduction velocity between any of the groups examined. Some evidence of mild sensory neuropathy was noted in the styrene-exposed workers (decreased duration of sensory amplitude potential). However, these effects were comparable in the group with the very high and very low exposure to styrene and it is therefore doubtful that this difference was due to styrene. No significant differences were noted between test and control groups in the EEG recordings, apart from a slight increase in the occurrence of fast activity in the central precentral areas. Such effects were largely confined to those in the low dose group and therefore were unlikely to be related to exposure to styrene. Hence, there was no clear evidence of any styrene-related EEG effects in this study.

Workers at two GRP factories in Poland have been studied (Dolmierski *et al,* 1976). The study was poorly reported. Atmospheric styrene levels at the factory were stated to be up to 71 pm (no further clarification given). Two groups of workers were formed, based on their age and duration of exposure. The largest group consisted of 43 young persons (age range 17-25) with relatively short duration of styrene exposure (average 1 year). The second group comprised 18 older workers (average age 42) with an average duration of exposure of ten years. There was no control group. Each worker was subjected to psychological and neurological examination, including an EEG recording.

Abnormalities in the EEG tracings were noted in 72% of the younger workers. These consisted mainly of discharges of sharp waves and high voltage slow waves in the temporal region. Most of the affected workers complained of neurotic symptoms and neurological examination revealed alterations in the deep reflexes, vestigial nystagmus and hand tremors. Considerably less marked effects were noted in the older workers, with abnormalities in the EEG tracings occurring in 22%. These consisted of low or medium voltage slow theta waves. Symptoms reported by the affected persons in both groups included fatigue, weakness and drowsiness. It was suggested by the authors that these symptoms and the EEG abnormalities were consistent with styrene-induced disturbances in hypothalamus function. However, in the absence of a control group and from the pattern of the results, it is unclear whether or not any of the observations made were a consequence of styrene exposure.

An increased incidence of abnormal EEG recordings has also been noted in a poorly reported study in workers exposed to styrene in Czechoslovakian factories (Klimkova-Deutschova *et al,* 1973). These were said to be most marked at one factory where ventilation and general hygiene conditions were known to be poor. However, no further details were available on the styrene levels present. A review of the case histories of 105 individuals apparently revealed increased irritability, fatigue, need for sleep, and symptoms of pseudoneurosis in addition to the pathological EEG recordings. An increased incidence of symptoms and abnormal EEG tracing was noted in workers exposed for a further three years. This was observed particularly with the EEG tracings; the abnormalities noted consisting of disturbances in background rhythm, flattening of the pattern, and more frequent occurrence of sleep activity. It was stated that there was a tendency for the effects to be related to urinary mandelic acid levels but no further details were given. Due to the lack of information on methods used and results obtained, it is not possible to make a conclusion from this study report.

The results of a neurological examination of 122 workers (27 male, 95 female) exposed to styrene at four Czechoslovakian factories have been reported (Hruba *et al,* 1975). No data were available on the atmospheric styrene levels. The average age of the workers was 35

(17-61). The reporting is unclear and there is no control group for comparison. Drowsiness was reported by 34% of workers and tiredness by 25%. "Cephalitis" was said to be present in 28%; this seems to mean that the subjects reported headaches. Examination findings included signs of disturbed balance in 64% and "disorders" of various reflexes in 20%. Other signs, of uncertain meaning, were noted with less frequency. EEG examination of 101 of the exposed workers revealed "marginal" effects in 42% ("flat" graphs, marked sleep abnormalities) and "slight but eventually average", abnormalities in 46% (principally synchronic rhythms). However, examination of a further 21 workers prior to employment showed "marginal" effects in 33% and some abnormalities (increased slow activity and tendency to synchronisation) in a further 33%. A slightly higher incidence of abnormalities was noted in this group three years after employment; at this time "marginal" effects were noted in 57% and abnormalities in 38%. A "normal" pattern was noted in only one individual at that time. These investigators therefore report almost two-thirds of pre-employment EEGs as departing from normal and more that 90% departing from normal following exposure, clearly indicating that they have a very low threshold of "abnormality". From examples in the paper the "flat" graphs to which the authors attach significance are simply very low amplitude recordings which could be caused by poor technical methodology. The poor reporting, questionable methods and lack of an adequate control group means that the results should not be accorded any significance.

Summary of electroencephalographic studies

Several studies have been conducted on EEG patterns in styrene-exposed workers. Overall, the collective findings do not provide robust evidence for the absence or presence of styrene-induced EEG changes in exposed workers. Furthermore, although the approach is valuable in that it provides measures of nervous system function that are independent of the level of collaboration of the subject, no clear criteria for interpretation of the health significance of any EEG changes that might have occurred are available. If styrene was the causal agent of EEG changes in workers covered by these studies, the most likely interpretation is that the effects were due to the general CNS depressant action of styrene.

Nerve conduction studies

In a large nerve conduction study, examinations were carried out on employees exposed to styrene at a US factory involved in the manufacture and polymerisation of styrene (Lilis *et al,* 1978). Clinical examination was performed, a symptom questionnaire completed and occupational and medical histories obtained from 494 styrene-exposed workers. The authors did not specify whether the survey covered all exposed workers at the plant. Following a review of job history, job description, technological changes made and results of a NIOSH evaluation, each worker was placed in a "high" (total 182) or "low" (total 306) styrene exposure category. No details of the nature of these exposure categories were provided; the exposure subdivision was used in some, but not all of the analyses. "Acute prenarcotic symptoms" were reported by 13% of the total number of exposed workers; 10% in "low" and 19% in "high" categories respectively. Workers with diabetes or high alcohol consumption were excluded from the analysis of findings from physical examination. In such examinations, in 5% (24 individuals) of the total number of employees there was evidence of distal hypoaesthesia (touch and pain) of the lower extremities, the incidence increasing to 9% in a group with >20 years of styrene exposure. However, it is not known whether this was a significant finding as no control group was investigated, no general population rates for these findings were presented and no attempt was made to adjust for the effects of age. Radial nerve conduction velocity was measured in 88 workers, and peroneal nerve conduction velocity in 91. After subjects with diabetes, back injury or significant alcohol intake were excluded, results from 80 and 73 workers respectively remained in the analysis.

Radial nerve conduction velocity was less than 55 m/s in 19% of the cases tested, and peroneal nerve conduction velocity was less than 40 m/s in 16%. The respective values of 55 m/s and 40 m/s were presumably lower limits of “normality” for the two nerves in the laboratory concerned but no justification for these cut-off values was given. There was a tendency for peroneal nerve conduction velocity to decrease with increasing duration of exposure but the effect was not statistically significant, and again no attempt was made to take account of the increasing age of the workers with longest exposure. In addition, where the group was divided on the basis of relatively “high” exposure and relatively “low” exposure, no significant difference in conduction velocity was noted between these groups. The lack of any control group is a problem in this study. The results do not offer convincing evidence in either direction, in relation to the potential of styrene to affect nerve conduction.

In another study, sensory and motor nerve conduction was measured in 70 men selected to cover all those at 50 ppm or above, and also in a sample of workers with appreciable lower exposure, at 4 GRP factories in Canada (Cherry and Gautrin, 1990). Exposure levels were obtained in environmental surveys (static and personal samples but no further clarification is given in the report). Mandelic acid concentrations in urine were also measured. Both atmospheric and metabolite levels were used as measures of exposure in the analysis. Workers had been exposed to styrene for between a few weeks and 20 years. After refusals, those with very short exposures, women, and those absent or not available were excluded. Data were obtained for 59 of the 70 men. Alcohol consumption data were obtained and the authors addressed potential effects of confounding factors in the reporting of the results. Eight workers reported tingling and pain. Neither the number of symptoms reported nor the number within a symptom type were related to exposure indicators. No relationship was found between motor nerve conduction velocity and any measure of styrene exposure. There was a dose-related statistically significant decrease in sensory nerve conduction velocity. All the results represented $\leq$10% difference in conduction velocity. The authors state that the only significantly “abnormal” results were in 2 workers where sensory nerve conduction velocity was “>2 standard deviations above the mean” when compared with normal values held at Montreal Neurological Institute. The report does not specify what level of exposure was identified for these 2 workers. Overall, this study demonstrated an exposure-related decrease in sensory nerve conduction velocity, the magnitude of the decrease being 10% or less.

A survey of nerve conduction velocities was conducted in workers from 3 GRP factories in Japan (Yausa *et al,* 1996). Workers were selected on the basis that they had been exposed to styrene in lamination work for at least a year, that information was available on work conditions, smoking habits and alcohol use and subjective symptoms, that they were not exposed to vibration or neurotoxic substances, and that they had no history of alcohol/drug abuse, diabetes or neurological disease. As a result, 9 workers were excluded and 32 exposed workers were entered into the study. Airborne styrene and personal dosimetry (for a few workers only) “short-time” assessments were performed. Urinary samples were collected on a Monday afternoon for mandelic acid measurements. Control workers were recruited from clerical jobs and matched for age (within 5 years) and sex. Four of the exposed group were not matched with a control; these were in the lower mandelic acid level subgroup (group A – see below). Electromyography was carried out on motor (ulnar and peroneal) nerves and sensory (ulnar and sural) nerves. The report does not describe when the tests were conducted. Styrene concentration varied according to task and reached 95 ppm in the breathing zone (reference period not stated). There were no significant differences between matched pairs of exposed and control workers in the reporting of peripheral neurological symptoms or any neurophysiological parameters. Exposed workers were divided into 2 subgroups; those with mandelic acid levels <250 mg/l (14 men) (A) and those with levels $\geq$250 mg/l (13 men) (B). Excretion of mandelic acid is related to very recent exposure to styrene, as styrene is not retained in the body for more than a few days. No significant difference was found in age, height, drinking habits and skin temperature between

groups A and B and the control group. Alcohol consumptions per week was greater in the lower mandelic acid group. There were no differences in maximum nerve conduction velocities of any nerve between groups A and B. Between group B and controls, statistically significant decreases were noted in motor nerve conduction velocities but there was no difference in sensory nerve conduction velocities. Longer motor nerve distal latencies were also found in group B. This study indicated a relationship between contemporary styrene exposure and decreased motor nerve conduction velocity.

A careful Japanese study examined nerve conduction parameters in a group of 11 male styrene-exposed workers and in 11 male unexposed controls matched by age (Murata *et al*, 1991). Exposure duration was on average 5 years. Exposure and medical histories were free from confounding factors such as vibration exposure and neurological disease. There were no significant differences between controls and exposed subjects for alcohol consumption, skin temperature and height. Airborne styrene concentrations 3 months before the study were stated to be on average 30 ppm, although no further details were given and hence it is not clear what this average represents. Electrophysiological studies were carried out during work hours (on a Tuesday and Wednesday) for the exposed group. Testing of the control subjects was done over a period of 2 years following the testing of the exposed workers. Tests included sensory and motor nerve conduction velocities and somatosensory evoked potentials. Seven workers reported one or more subjective symptoms such as local irritation, fatigue and headache. The variability with time in electrocardiographic R-R wave interval (variation in heart rate), a very indirect measure of autonomous nervous system function, was statistically significantly lower (3.5% (2.2-6.1)) in the exposed group compared with the referents (5.5% (1.8-9.6)). This change in the direction of less variability does not have clinical significance; physiological variations in heart rate of no functional significance occur in normal subjects. Sensory nerve conduction velocity (55.1 m/s (51.3-60.0) in the exposed group, 56.9 (49.2-60.0) in the referent group) was statistically significantly lower in the exposed group compared with the controls and the distribution of nerve conduction velocities (v80 velocity, below which 80% of fibres lie was 60.5 (53.2-67.8) in the exposed group and 63.3 (59.0-69.5) in the referents) was interpreted as showing that the distribution of fibres was shifted towards the slower velocities in the styrene exposed group when compared with the controls. No significant difference was found on motor nerve conduction velocity, on somatosensory evoked potential or on heart rate. It is not known what biological significance the change in distribution of nerve conduction velocities might have; thus the main finding of this study is the slightly (3%) lower sensory nerve conduction velocity in exposed workers compared with controls. However, given the very small number of subjects invetigated and the potential bias introduced by testing the controls in a different environment over a very different period, the interpretation of this finding is rather difficult.

Nerve conduction measurements were obtained in a study of 20 Czechoslovakian workers (5 male, 15 female) exposed to styrene and a group of 30 male and 40 female controls (Stetkarova *et al,* 1993). The main age of the exposed workers was 38 (men) and 45 (women) and that of controls 31 and 32 years respectively. Workers were exposed for an average of 11 years. The controls had no history of exposure to neurotoxins or neurological disorders. Alcohol intake is reported in bands based on divisions of 20 mg/day and 50 mg/day. These must be reported in error as 20 mg alcohol is less than one millilitre of bitter beer. It is possible that the numbers refer to g/day. “High” alcohol consumption (>20 “mg”/day) was noted in 4 styrene-exposed workers but it was stated that “alcohol intake did not correlate with response to styrene”. Some exposed workers reported symptoms of sleep disturbance, headache, fatigue and forgetfulness. Finger and eyelid tremors, hyper-reflexia, hyperhidrosis, decreased Achilles tendon reflex (10% of group) and slight carpal tunnel syndrome (15% of group) were found in the exposed group. The carpal tunnel syndrome was thought to be associated with work patterns, as the styrene-exposed subjects also reported other musculoskeletal disorders. No indication was given of reporting of symptoms in control subjects. “Mean year round” styrene levels were 33 – 136 ppm in the breathing

zone. There were no specific details reported of how this average was calculated. Evoked responses were analysed after electric stimuli were delivered to the median and tibial nerves. Peripheral nerve conduction velocities and central conduction time after tibial nerve stimulation were statistically significantly decreased in the exposed group compared with the control group. Significantly increased latencies of peripheral and cortical somatosensory evoked potentials (sep) to median nerve stimulation and cortical seps to the tibial nerve were observed. All the measured differences in velocity and latency were less than 10% of the control group value. Height was a major factor in the variation of seps but it is not clear from the report whether height was adjusted for in the analysis, as the methods are not fully described. The results for 6 exposed workers were classified as “abnormal” (falling outside of 95% of the reference range). There was no significant correlation between abnormal findings and duration of styrene exposure. The authors also considered that the abnormal findings for the median nerve could be due to carpal tunnel syndrome caused by daily excessive strain on the right hand. Nevertheless, the results showed that nerve conduction velocity in styrene- exposed workers was slightly (<10%) lower than in a comparable control group.

In another study involving a small group of 11 male workers (total exposed not reported) in Germany, the effect of styrene exposure on nerve conduction measurements was investigated (Triebel *et al,* 1985). A control group of 11 healthy men of similar age was included. The control subjects had no known exposure to neurotoxic agents or situations and were not occupationally exposed to styrene. None of the exposed workers had histories of alcohol abuse, diabetes or drug use. They were exposed for a median of 4 years. Mean personal exposure was 92 – 114 ppm (8-hour TWA) measured on 3 days. No difference between the two groups was observed on motor and sensory conduction velocities in the ulnar or median nerves. Since this study was small the negative results obtained are not definitive in themselves. The results of a neurological survey of 98 workers exposed to styrene at 24 GRP factories in Finland have been reported (Seppalainen and Harkonen, 1976; Harkonen, 1977; Harkonen *et a,* 1978a and 1978b). Peripheral nerve conduction velocity was measured in 40 of these men; these were individuals that reported most “subjective complaints”. The symptoms being reported were not described in the study report. The mean age of these exposed workers was about 30 years and the mean period of exposure five years. None of the exposed workers had diabetes, epilepsy, or history of unconsciousness of more than 30 minutes duration. The 30 healthy male control subjects were of a very similar age distribution and had no occupational exposure to neurotoxic substances. No details of the atmospheric styrene levels were given, but post-shift urinary mandelic acid concentrations were monitored in each worker once a week (on different days) for five weeks. The median urinary mandelic acid concentration was about 850 mg/l and the range 30-3811 mg/l. The nerve conduction investigations consisted of determining maximum motor conduction velocity of the median, ulnar, deep peroneal and posterior tibial nerves, sensory conduction velocity of the slower fibres of the median and ulnar nerves and also conduction velocity of the slower motor fibres of the ulnar and deep peroneal nerves. The nerve conduction velocity results of the exposed group did not differ from those of the age-matched control group. The authors identified “slightly abnormal” results, which could not be accounted for by known etiology in exposed workers. However, these workers were found to have low mandelic acid levels. Hence, nerve conduction velocity in styrene-exposed workers was similar to unexposed controls in this study.

Vibration and thermal threshold tests were conducted to test for effects on the peripheral nerves in a group of 86 workers from 6 GRP plants in Taiwan (Tsai and Chen, 1996). Although 177 were initially entered into the study, some refused and some failed to complete the tests and others were excluded for reasons such as alcoholism. Information was gathered on smoking history and other lifestyle factors and subjects were asked to complete subjective symptom, occupational and medical questionnaires. A vibramater was used to determine the threshold of detection of vibration on the left index finger and big toe on each

worker. Detection of a change in temperature delivered by a thermode on the left forearm was also recorded. All the tests were administered away from the workplace and at least 16 hours after last exposure. Exposure to styrene (personal and area sampling) was measured on typical work days. Using 8-hour measurements, a non-exposed group of 45 workers (mean 1.0 ppm) and exposed group of 41 workers (mean 21.9 ppm) wee identified. Multiple regression analysis was applied “to control for possible confounding factors” of age, years of education, gender and alcohol consumption. Numbers of reported symptoms were not significantly correlated with styrene exposure. Only the vibratory threshold test results were significantly positively associated with styrene exposure. However, vibration perception has been used rather little in styrene workers and has the disadvantage that it might be affected by a low motivation to perform well.

GRP workers exposed to styrene in Canada were studied for sensitivity to vibration and touch using a test battery related to sensory function (Mergler *et al,* 1992). From a total of 151 workers employed in 3 plants, 136 (91%) volunteers were recruited for the exposure assessment part of the study. Of the 128 (85.6%) workers that agreed to participate in the performance and function testing, 11 were excluded for reasons which included less than 6 months work at the plant, history of exposure to neurotoxic substances and head injury. Each participant was questioned about work history, smoking habits and alcohol consumption. Testing was administered 12 hours after the last exposure. Vibration testing appeared to be a requirement to detect vibration in the fingers/toes but the methods are not given in any detail. The exposure assessment results were reported separately (Truchon *et al,* 1992). Personal air sampling data were gathered throughout a shift and post-shift urinary mandelic acid measurements obtained for each worker. The highest 8-hour TWA arithmetic mean exposure levels of 131, 120 and 117 ppm were found in the chopper-gun operator (moulding), painters and laminators groups respectively. The same groups had mandelic acid levels of 0.73, 0.56, 1.26 mmol/mmol creatinine. The workers were divided into two groups according to urinary post-shift mandelic acid (>0.6 or <0.6). The groups were similar in age, level of education and alcohol consumption but the higher metabolite level group had been employed for longer. Touch and vibration sensitivity in the extremities was the same in the two groups.

Summary of nerve conduction studies

The available nerve conduction studies have produced inconsistent results for different groups of workers exposed to similar levels of styrene. Some studies have indicated a correlation between styrene exposure and small (<10%) decreases in nerve conduction velocity, compared with unexposed controls. Others have shown similar nerve conduction velocities in styrene-exposed and unexposed workers. Overall, it is not clear if styrene exposure can produce a small decrease in nerve conduction velocity; furthermore, if it can, the underlying basis for the effect could not be deduced from the available information. Also, the clinical significance of this effect is questionable, as all subjects appeared to be healthy workers.

Otoneurological and audiometry studies

An otoneurological study was carried out on a small group of GRP workers (Calabrese *et al,* 1996, De Rosa *et al,* 2000). Twenty workers were employed in 3 plants. It is not stated whether all the available exposed employees participated. The choice of tests was aimed at investigating any effects of styrene on the vestibular system or hearing. The workers were stated to have relatively low alcohol consumption. Exposure levels were measured in the workplace on the day before otoneurological testing. Average personal exposure levels (8-hour TWA) were 36 $\pm$ 20 ppm for styrene and 65 $\pm$ 28 ppm for acetone. Concentrations of

urinary metabolites were also obtained. Audiometric tests and auditory brainstem responses (ABR) were measured and vestibulocular (VOR) (caloric and rotation) and vestibulospinal (VSR) (static posturography) tests performed. Nine of the subjects were also examined after an exposure-free period of 3 weeks. All workers had a normal hearing threshold. The mean ABR parameters (e.g. latencies) were not significantly different between the exposed group and an undefined control group. “Abnormal” results were obtained for 17 of the 20 workers in the VOR caloric test and in 14 of 20 in the rotation test. However, no criteria for defining “abnormal” were provided and no controls were tested for comparison. The postural test results for 8 exposed workers were no different from those of an undefined reference group (n=84); there were no significant changes in the worker test results after 3 weeks without exposure. The only test in which some improvement occurred (in 5 subjects) after the exposure-free period was the caloric test. Overall, there were no effects of styrene exposure on the auditory system in this study although it is noted that there was a relatively small number of workers investigated. A high incidence of undefined “abnormalities” in certain reflexes related to the vestibular system was noted among the styrene-exposed workers, but in the absence of better characterisation of the results and the lack of a control group for comparative purposes, this result is not convincing.

In Sweden, a small factory using styrene was closed and the workers, employed on average for 10.8 years, were no longer exposed to styrene (Moller *et al,* 1990). The time lapse since last exposed was not reported but it appears that the same ex-workers were studied by Flodin *et al,* 1989), 7-9 months after exposure ceased. Generally, measurement of airborne styrene in the work areas had been performed annually. The measurements were obtained by personal sampling and concentrations refer to “eight hour average”. “High” exposure levels had been 12-24 ppm with a very few peak exposures above 71 ppm. An “otoneurological” study was carried out, using highly specialised tests, to examine the hypothesis that styrene exposure may affect central nervous system mechanisms relating to balance and hearing. The total workforce figure was not given but appears to have been 24 (Flodin *et al,* 1989). The 18 participating ex-workers (mean age 40) were matched for age range and alcohol consumption with 18 controls in solvent-free employment. Also, static posturography was measured in the ex-workers and in a control group of 52 men (mean age 48) from the construction industry with no exposure to solvents.

Clinical neurological assessments in the styrene-exposed workers (gait, tendon reflexes, gross motor function and cranial nerves) gave normal findings. In auditory tests, a slight high-frequency hearing loss was detected, but this was accounted for by age and/or excessive noise exposure. Abnormalities in cortical response audiometry, defined as a latency above the normal reference range for the response to a frequency glide stimulus, were found in 7 ex-workers. There were no apparent effects of styrene exposure in the vestibular stimulation test by caloric irrigation of the ears. Postural sway area on a forceplate was considered by authors to be abnormally large, relative to controls, in 10 ex-workers. The actual mean sway area was small (increased to 117 mm^2 compared with 71 mm^2 in controls). There was a statistically significant increase in saccade (quick movements of the eyes) latency time and impairment of ability to visually suppress vestibulocular reflec (rotatory test) in the styrene-exposed ex-workers compared to matched controls. The actual change in mean latency was an increase from 249 to 289 ms. The cortical response “abnormalities” detected in the absence of any effect on hearing, are not considered to have any clinical significance. However there was some evidence for an effect on vestibular function in the exposed workers, although the findings are not particularly robust.

Workers in an industry in the Netherlands were exposed to styrene; 59 male subjects of 76 employed were included in a study of hearing function at up to 16 kHz (Muijser *et al,* 1988). Illness prevented 12 taking part and 3 others refused. A control group of 94 male photographic film workers were of similar age and were stated to be “not occupationally exposed to styrene or other chemicals”. A medical history of hearing dysfunction led to

exclusion of 2 exposed and 6 non-exposed workers. The concentration of styrene in the breathing zone of some of the workers was measured on 3 consecutive days using personal absorption badges. Workers were divided into two groups according to whether they were directly (n=31) or indirectly (n=28) exposed to styrene. A task was chosen to represent "peak" exposure to styrene and measurements made while this task was conducted. The "4-hour mean" exposure levels were 32 ppm (directly exposed) and 14 ppm (indirectly-exposed); "peak exposure" was 105 ppm. The workers had been exposed for an average of 8.6 years. Noise levels at the styrene workplaces were up to 70 DBA in the background and up to 104 DBA for short periods, at which level noise-induced high frequency hearing loss is possible. Noise exposure at the control workplace was 80-85 DBA. Direct comparison of results in the styrene-exposed and non-exposed workers showed a higher threshold for high frequency hearing in the styrene-exposed group but on multivariate analysis to adjust for the effect of age, the difference was not statistically significant. There was also no statistically significant difference between the control and directly-exposed group. However, hearing thresholds at high frequency were statistically significantly higher in the directly-exposed versus indirectly-exposed subgroups workers. A higher threshold would be expected at higher frequencies if styrene exposure were to have an adverse effect; high frequencies are known to be more sensitive to impairment. On detailed analysis, the authors describe an inconsistency in the pattern of results with respect to this expectation; a significant effect was detected at 8 kHz but not at higher frequencies. Hence the authors stated that their findings were equivocal. It is noted that Muijser *et al* failed to take noise exposure into account. This is a significant flaw in the design of the study. The pattern of results is not convincing in terms of an association between an effect on hearing and exposure to styrene; neither is this study a satisfactory test of the hypothesis that occupational exposure to styrene causes an adverse effect on hearing.

In another study, the possible association between styrene exposure and hearing loss was investigated (Sass-Kortsak *et al,* 1995). All male workers at 14 Canadian GRP plants and at one office workplace with no solvent exposure were asked to participate. From a total of 324 approached, nine workers were excluded on the grounds of medical conditions potentially affecting hearing; 16 refused to take part. The workers were interviewed in order to obtain occupational and smoking histories and history of occupational or recreational noise exposure. Audiometry was performed at up to 8kHz at the start and end of a workshift and personal noise and full-shift styrene exposure measurements made on the same day. The men were divided into high, medium and no styrene exposure groups according to work categories. The styrene concentrations were 14 ppm and 3 ppm, 25 ppm and 8 ppm, and 0.4 ppm and 2.5 ppm geometric and arithmetic TWA means in the high (n=70), medium (n=8) and no (n-43) exposure groups respectively. No reference period was specified for the calculation of these averages. The ages of workers in these groups were similar. A measure of cumulative exposure was constructed. Experienced occupational hygienists assigned lifetime styrene exposure figures and noise exposure estimates. Over the course of a shift, results for individual workers showed equal likelihood of enhancement in hearing acuity as of reduction compared with values at the beginning of the shift. Regression analysis was carried out, excluding those above 50 years of age because of the strong effect of age on hearing. Age remained a highly significant variable. In this analysis, lifetime styrene exposure was not a significant factor in hearing loss. Therefore styrene exposure was not associated with adverse effects on hearing in this study.

In Japan, 115 male workers exposed to styrene in various workplaces were recruited for a study of the upper frequency threshold of hearing, which the authors of the study believed to be a more sensitive indicator of early hearing loss than conventional audiometry (Morioka *et al,* 1999). In many cases they were simultaneously exposed to other solvents including toluene. On the basis of pre-existing impaired hearing, exposure to intense noise or incomplete data, 18 workers were excluded. Sound levels measured at the workplace were 53-95 DBA. Styrene was measured in the breathing zone and levels of urinary metabolite

mandelic acid also measured. Styrene levels were found to be generally less than 50 ppm TWA (range 0.1-100 ppm). Conventional audiometry at 0.5-8 kHz, performed pre-shift, demonstrated no significant difference between the exposed subjects and controls, described as "the subjects for the standard upper limit age curves for males". (It is not clear where this "standard" data was obtained, though it appears likely that it is from previous studies by the same authors.) The upper frequency limit of hearing was determined for each exposed subject and plotted on a set of "standard" percentile curves for male upper limit of hearing vs age. Subjects whose upper frequency threshold fell below the 75th percentile curve were defined as "cases" of hearing loss, and most of the subsequent analyses are based on the percentage prevalence of such "cases". In a plot of the prevalence against duration of exposure the prevalence exceeded 25% (the expected "normal" value) at 5 years of exposure and increased linearly to 35% at 10 years of exposure, after which it remained static. For the 54 workers exposed for more than 5 years there was a significant correlation between "individual percentiles" (presumably individual results expressed in terms of position on the normal percentile curves) and styrene exposure. The prevalence of cases (defined as above) also increased with styrene exposure, becoming significantly higher than 25% when exposure exceeded 16 ppm. When analysed with respect to mandelic acid levels the prevalence of "cases" significantly greater in the group whose level exceeded 0.3 g/l compared with those below that level. There was evidence of a correlation between styrene exposure (concentration and duration) and abnormal results in a test for the upper frequency threshold of hearing. However, these subjects were also exposed to relatively high noise levels. Hence, no conclusions can be drawn about the role of styrene in these findings.

Morata et al. (2002) investigated potential auditory effects produced by occupational exposure to low levels of styrene and noise. Workers (n = 154) exposed to styrene were recruited from factories producing fibreglass products. Sixty-five were not exposed to potentially harmful levels of noise; the remaining 89 styrene-exposed workers also had noise greater than 85 dB(A) L_{eq} over an 8 hour workday. A noise-only group comprised 78 metal workers, and 81 postal workers formed the control group, without exposure to noise or styrene. Each subject answered questions concerning occupational history, medical history and lifestyle factors. These data were combined with measurements of exposure to styrene and to noise in current employment, estimates for styrene and noise exposure over each subject's lifetime, and hearing thresholds given during the study. Styrene exposure was determined by air monitoring and biological monitoring. Air from the breathing zone of each subject was sampled over the test day. Mandelic acid (MA) was measured in urine from 127 of the 154 styrene-exposed workers; samples were collected over 24 hours from the beginning of the work shift under study. Styrene concentrations in air were reported only in a scatterdiagram together with MA excretion in urine. Although the correlation was statistically significant ($r = 0.27$, $p < 0.001$), there was a great variability. Noise exposures were determined as 8-hour dB(A) L_{eq} values for 185 of the workers; the remaining 128 subjects were assigned the mean exposure of other workers doing the same tasks. Each worker's previous noise exposure was estimated from questionnaire responses or database information. Using the measured or assigned exposure for current employment, and the approximate values for previous employment, lifetime noise exposure was estimated as an 8-hour L_{eq} over each working day. For the noise only group, the current exposures ranged from 75 to 116 dB(A) L_{eqbh}. An unstated number of this group did not receive "excessive" noise, above 85 dB(A) L_{eqbh}, and therefore could have been placed in the control group. The styrene only subjects had a range of current styrene exposures from 0.05 ppm to 23 ppm, while for the styrene and noise group, styrene ranged from 0.007 ppm to 12 ppm. Such wide ranges of noise and styrene measurements suggest that the exposure groups were not rigorously purified. Each subject gave an audiogram which was evaluated for hearing loss at 1, 2, 3, 4, 6 and 8 kHz. An audiogram was considered "normal" if no threshold exceeded 25 Db Hearing Level at any frequency. A "high-frequency hearing loss" was noted if the thresholds were poorest in the frequency range 3 to 6 kHz. Over the 4 exposure groups,

prevalence of high-frequency hearing loss ranged from 33% to 48%; the differences between groups were not statistically significant.

The binary hearing variable, normal versus high-frequency loss, was also employed in a further analysis. The medical, lifestyle and occupational data (including current and past exposure to noise and to styrene) were subjected to a multiple logistic regression analysis to estimate the odds of subjects developing a high-frequency hearing loss. Only age, current noise exposure and MA in the urine were significant. Neither current nor lifetime styrene exposure achieved statistical significance. The authors concluded that exposure to styrene, at concentrations below 20 ppm, produced high-frequency hearing losses. However, considering the unclear information on styrene exposure of the subjects and the lack of dose-response relationships, this conclusion is not substantiated by the available evidence.

In a cross-sectional study of 299 workers, who had each been employed for more than 6 months at either of 4 yacht-making yards and one plastics factory in Poland, visual examinations of the ear, nose and throat, and audiometric tests were conducted (Sliwinska-Kowalska *et al*, 2003). Workers were excluded from the study if there was evidence of previous ear disease, impaired hearing, or other non-occupational risk factors for hearing loss; 9 were excluded from the original cohort. Pure-tone audiometry was performed on 290 workers (aged 20-60). Occupational noise exposures were assessed over a working day in each subjects' curent employment. Those with an 8h average exposure greater than 85 dB(A) were defined as noise-exposed; those with lower noise exposures were assigned to the no-noise cells of the experimental design. Hence, of the 290 workers, 194 were listed as part of a 'styrene-only' cohort (i.e. exposed to styrene and to a mean background noise level of 80 dB(A)); 26 as exposed to 'styrene and toluene' (i.e. exposed to styrene, toluene and to a mean background noise level of 80 dB(A)); 56 to 'styrene and noise' (i.e. exposed to styrene and to a mean noise level of 89 dB(A)); 14 to 'styrene, toluene, and noise' (i.e exposed to styrene, toluene and to a mean noise level of 89 dB(A)). Chemical exposures were mixed, and more than 80% of the workers in the 'styrene-only' cohort were exposed to other solvents (including toluene, dichloromethane and acetone) at levels that exceeded the OELs in Poland (100 mg/m^3, 50 mg/m^3, and 200 mg/m^3 respectively). Peak levels (unclear if these were 8-hour TWA values) were 225 mg/m^3 for toluene, 307 mg/m^3 for acetone, and 145 mg/m^3 for dichloromethane. An age-matched control group of 157 'white collars' and 66 workers from a metal factory was selected with similar exclusion criteria. Of this 'control' group, the 66 metal factory workers were exposed to mean noise levels around 89 dB(A) (noise only control group) and the 157 'white collars' to a relatively low mean noise level of 73 dB(A) (no noise, no chemicals control group). Current airborne exposure to styrene was assessed by personal monitoring during a working shift. The concentrations ranged up to a maximum of 47 ppm. A lifetime-average styrene exposure was calculated for each of the 290 chemical-exposed group, using environmental inspection records over the previous 15 years. These averages ranged from 4 to 309 mg/m^3 (mean 62 mg/m^3; 1-71 ppm, mean 14 ppm). Lifetime estimates were not presented for any of the other chemicals present in the workplace.

Abnormal audiograms were seen in about 63% (183/290) of the solvent-exposed workers compared to around 40% (93/223) of controls. The odds-ratio for hearing loss (based on abnormal audiogram) likely to be due to styrene exposure but also to exposure to a higher mean noise level was 5.2 (95% CI 2.9-9.8); this was calculated comparing the incidence of the abnormal audiograms in the styrene-only group (mean noise level of 80 dB(A)) with that in the cohort not exposed to any solvents but to mean levels of noise of 73 dB(A). The odds-ratio for hearing loss due to noise only was 3.4 (1.7-6.4); this was calculated comparing the incidence of the abnormal audiograms in the 'styrene and noise' group (mean noise level of 89 dB9A)) with that in the styrene-only group (mean noise level of 80 dB(A). The odds-ratio for hearing loss due to exposure to styrene and toluene but also to a higher mean noise level was 13.1 (4.5-37.7); this was calculated comparing the incidence of the abnormal audiograms in the styrene and toluene group (mean noise level of 80 dB(A)) with that in the

cohort not exposed to any solvents but to mean levels of noise of 73 dB(A). The odds-ratio for hearing loss due to exposure to styrene and noise was 10.9 (4.9-24.2); this was calculated comparing the incidence of the abnormal audiograms in the styrene and noise group (mean noise level of 89 dB(A)) with that in the control cohort not exposed to any solvents but exposed to a low mean level of noise of 73 dB(A). The odds-ratio for hearing loss due to exposure to styrene, toluene and noise was 21.5 (5.1-90.1); this was calculated comparing the incidence of the abnormal audiograms in the styrene, toluene and noise group (mean noise level of 86 dB(A)) with that in the in the control cohort not exposed to any solvents but exposed to a low mean level of noise of 73 dB(A). Hearing threshold was also increased amongst styrene-exposed workers when compared to controls, and was greater when in combination with other solvents or noise.

This study shows some evidence of styrene-induced hearing loss and an additive or synergistic effect with other ototoxic agents such as toluene or noise. However, since in the 'styrene only' group, workers were also exposed to relatively high levels of noise (around 80 dB(A)), it is difficult to assess whether the effect seen was due to styrene exposure or to noise or both. Furthermore, interpretation is hampered by co-exposure to other substances (dichloromethane and acetone).

Summary of otoneurological and audiometry studies

A few studies are available which were designed to investigate any effect of styrene on hearing function. When confounding factors such as age and noise exposure were taken into account, no relationship between styrene exposure and hearing loss was found. There is limited evidence that styrene exposure may have caused minor effects on vestibular reflexes in some workers. However, the quality of the exposure data is such that it is not possible to relate these effects to reliable levels of styrene exposure. Therefore, although these human data cannot be used for risk characterisation purposes, nevertheless they indicate that the observations of ototoxicity in animals may be relevant to humans.

Studies on olfaction

One study which investigates olfaction in styrene-exposed workers is available (Dalton *et al,* 2003). Odour detection thresholds and odour identification ability were investigated in styrene-exposed workers in the GRP industry. Workers were selected from four facilities. Medical and occupational history questionnaires were used to identify eligible workers, of whom all except 4 agreed to participate in the study. Exclusion criteria included relevant pre-existing medical conditions and exposure to chemicals having known or suspected adverse effects on olfaction. A total of 52 exposed workers took part in the study (mean age 37.6 years, mean duration of exposure 12.5 years, 35 smokers); a group of 52 unexposed workers, matched for age and gender, and with similar socio-economic status and geographical location was also included (mean age 36.7 years, 27 smokers). Five individuals in each group were aged 50-60 and all others were under the age of 50; this should have limited the impact on this study of the normal age-related decline in olfactory sensitivity generally observed above the age of 60. Serial dilutions (in an odourless solvent) of phenylethyl alcohol (PEA, a standard substance used as a measure of general olfactory function at this laboratory) and styrene were used in the assessment of odour detection. Subjects inhaled around 500 ml of air directly from a bottle containing styrene or PEA in a styrene-free laboratory environment, and, using a variety of different concentrations, were asked if they could identify the odour. Other substances used for retronasal odour function and nasal odour identification were inhaled from small open containers or plastic bottles (stimuli included odours such as banana, fish, and lemon). Workers were tested during their regular work shift, having worked full shifts on either one or two days immediately preceding the test day. Subjects refrained from smoking, eating or drinking for one hour prior to testing.

Personal exposure monitoring (current and historical) was performed for each worker in the study. Current exposure was assessed by personal air monitoring in the breathing zone and by analysis of urine for mandelic acid and phenylglyoxylic acid; measurements were performed on the one or two days prior to the test. An assessment of each worker's historical airborne exposure (ppm years) was also conducted (Lees *et al,* 2003), from historical exposure measurements and information on each individual's job title, and using biological monitoring information from urine samples. The highest airborne styrene concentrations (up to 77 ppm, 333 mg/m^3 8-hour TWA) were confined to three individuals although for each of these three workers personal exposure was mitigated by the use of respiratory protection. For other activities involving the wearing of RPE, the exposure estimate was adjusted by a factor of 5 to take account of the protection factor. Mean 8-hour TWA airborne exposure was around 12.5 ppm (54 mg/m^3). Current exposure measurements and constructed estimates indicated a uniform exposure over the previous 15 years, there being essentially no increase or decrease in mean values.

There were no statistically significant differences in the odour detection threshold for PEA, in performance in the retronasal test or in odour identification between exposed workers and matched controls. However, a statistically significant difference between exposed workers and matched controls was found for the odour detection threshold for styrene; the odour threshold was about 32-fold higher in exposed workers compared with the matched controls. Given that the other measures of olfactory performance showed no differences between workers and controls, the difference in the styrene odour detection threshold is most likely to be indicative of exposure-induced adaptation. The difference between workers and controls was most marked in workers in older age groups (i.e. aged 40-60), although this did not appear to be associated with an increase in duration of exposure. Exposed workers generally were more likely to report disturbances in olfaction, compared with controls (when assessed by self-reported questionnaire); however, the results of the olfactory performance tests do not indicate any olfactory dysfunction and thus it is possible that the workers' responses were due to the perceived loss in sensitivity to styrene odour in the workplace. The results for the workers were also compared with the expected scores for the general population; no notable differences in the results were found. Overall, therefore, this study indicates a reduction in sensitivity to styrene odour, most likely to be attributable to adaptation, with no evidence for any deficit in olfactory function in workers exposed to mean styrene levels around 12.5 ppm (54mg/m^3), 8-hour TWA and up to about 77 ppm (8-hour TWA).

Studies on colour vision

The data available on colour vision and styrene exposure have been recently reviewed by Lomax et al (2004). This section of the RAR reflects the interpretation/evaluation of the data presented in this review.

The most common test of colour discrimination is the Lanthony Desaturated 15-hue test (D-15d), which has been critically reviewed by Geller and Hudnell (1997). In this test the subject is presented with 15 coloured caps and asked to arrange them in a natural colour sequence, starting from a reference blue cap and successively matching each cap to the preceding one in the sequence. The theoretically correct sequence, chosen by most subjects, follows a spectral order from blue through green, yellow and orange to red and on to purple. The colours used are desaturated ("pastel" hues in everyday English language) which increases the difficulty and consequently the sensitivity of the test. The results can be analysed both qualitatively and quantitatively.

Qualitative analysis involves plotting the results on a circular diagram of the correct colour sequence by joining the dots corresponding to caps that the subject placed consecutively.

Errors appear as chords cutting across the circle. They can be classified according to whether they lie parallel to the red-green or blue-yellow axes of the circle and whether they are minor (transposition of adjacent caps) or major (spanning two or more caps). The frequency of errors is influenced by the intensity and colour of the lighting used for the test and by the visual acuity of the subject. Many subjects make minor errors in the test and a substantial number make one or more major errors, predominantly along the blue-yellow axis. The frequency of these errors increased with age. Congenital colour vision defects give very characteristic patterns of major errors of large magnitude on qualitative analysis, corresponding to the various clinically recognised forms of “colour blindness”. It is important to note that in the more recent studies on colour vision and exposure to styrene, individuals with congenital colour vision deficiency were excluded by screening with the Ishihara plate test.

Quantitative analysis is based on allocating numbers to each cap corresponding to its position in a standard chromaticity chart and calculating the “colour distance” between successive pairs of caps as placed by the subject. These colour distances are summed up to give the total colour distance score (TCDS), which can be divided by the theoretically perfect score for the set of caps used, to obtain a standardised score called the colour confusion index (CCI).

Twelve cross-sectional or longitudinal studies investigating colour discrimination in reinforced plastics workers, boat builders and other workers exposed to styrene have been identified.

A recent, well-reported study examined colour discrimination in styrene exposed laminators at a German boat-building plant before and after a 4 week vacation, with the assessment being repeated one year later after the introduction of improved hygiene measures (Triebig *et al*, 2001). Thirty out of 50 male styrene-exposed laminators who had been at the factory for at least 6 months were recruited into the study; it is unclear why the other 20 laminators did not participate in the study. Twenty-seven male workers from other parts of the factory were recruited as controls. Subjects were excluded from the study for a number of reasons including congenital colour vision deficiency (identified with Ishihara colour perception tables), high alcohol consumption (above 210 g/week), poor visual acuity, diagnosis of ophthalmological disease, and, in a control subject, high mandelic acid and phenyl glyoxylic acid (MA+PGA) levels. After exclusions, 22 styrene-exposed workers and 11 control workers entered the study. The median age of the styrene-exposed workers was 38 years, and the median time working at the plant was 4.5 years (range 1-21). The control workers were similar in terms of age, alcohol consumption and duration of employment. Styrene exposure was assessed by biological monitoring of MA and PGA. These were measured in urine collected at the end of the shift on a Thursday afternoon, in order to give a measure of the peak body burden of styrene. Colour discrimination was assessed binocularly by the D-15d test, conducted under standardised lighting conditions. Testing was carried out in exposed workers and in controls on Monday morning before starting work and on Thursday afternoon of the same week. CCI values greater than the 95th percentile of the age-dependent reference value (shown in Table b) were considered abnormal.

During the first phase of the study median MA+PGA levels were 472 mg/g creatinine (range 11-2399) in the exposed group and 15 mg/g creatinine (range 5-36) in the control group. Regarding colour discrimination results, on the Monday morning, 8 of the 22 exposed workers (36%) had abnormally high CCIs, compared with 2 of the 11 controls (18%). Although the nature of the test errors was not described in detail, both tritan and red-green errors were made. At the Monday morning time point the mean CCI in the exposed workers was 1.24 (SD 0.25), higher than in the controls (mean 1.10, standard deviation (SD) 0.11), but the difference was not statistically significant. For the Thursday afternoon test the mean CCI in the styrene-exposed workers had increased to 1.29 (SD 0.09). This was significantly

higher than in controls, for whom the mean CCI did not change between Monday morning and Thursday afternoon. Thursday results tabulated for 19 exposed workers showed that all 7 who had abnormally high CCI values had urinary MA+PGA concentrations exceeding 450 mg/g creatinine, whereas 9 of the 12 workers with normal CCI values had MA+PGA concentrations below 450 mg/g. When the data from all the styrene-exposed workers were analysed by regression analysis, CCI was positively and significantly correlated with the urinary concentration of MA, and with the sum of MA and PGA. After a 4-week absence from the factory, the mean CCI for the exposed workers was significantly lower than the pre-vacation values, and was no longer significantly different from the mean control CCI.

A re-examination of colour discrimination was carried out in the same exposed and control subjects one year later, following the introduction of an improved ventilation system at the plant. By the time of the second phase of the study styrene exposure had decreased, shown by lower urinary styrene metabolite levels among laminators. Measurements on Thursday afternoon showed that the median end-of-shift metabolite level was 273 mg MA+PGA/g creatinine, almost half of the level found one year previously. Colour discrimination tests carried out on Thursday afternoon showed that the mean CCI among the same group of laminators was significantly lower than one year previously, at 1.16 (SD 0.14). Five laminators (23%) were still classed as having abnormally high CCI values, although for the tests conducted on the same subjects on Monday morning only 2 styrene-exposed workers gave results outside the expected range. Among the 11 control subjects, there were no changes in colour discrimination in the year between the two phases of the study. After a 4-week absence from the factory, the mean CCI value for the exposed group fell to 1.05 (SD 0.06), almost identical to that of the control group.

This well-conducted, well-reported study shows a convincing association between occupational styrene exposure (as revealed by the concentration of urinary metabolites) and poor colour discrimination, with both blue-yellow (tritan) and red-green errors being made. From the design of the study, it is not likely that this association could be accounted for by confounding factors such as age, alcohol or congenital colour vision deficiency. On the basis of the ACGIH conversion, this would suggest that elevated CCI scores occur for exposures to styrene estimated to be between 20 and 30 ppm (8 h TWA). It is therefore unlikely from the biological monitoring data that exposures to styrene in this study would have been high enough to cause acute CNS depression. The results from both phases of the study consistently indicate that the effects of styrene on colour discrimination decrease after an exposure-free weekend, and appear to be fully reversible over a period of four weeks. The results also show an exposure-response relationship between CCI and urinary levels of MA and PGA. In terms of severity of effect, the authors considered the effect of styrene on colour discrimination to be mild, as the workers were said not to be consciously aware of any effect.

Italian studies

Three reports described a series of linked studies investigating colour discrimination in styrene-exposed workers at a number of reinforced plastics factories in Italy.

In the first study, 75 styrene-exposed workers employed at 7 factories were recruited from a total workforce of 84 (Gobba *et al*, 1991). The average length of time working with styrene was 7 years. Sixty workers from the rock-wool industry who were not exposed to solvents acted as controls. Two exposed workers and 3 controls were excluded from the study population on the basis of high alcohol consumption (>250g per week), poor visual acuity or a case history of certain medical conditions including self-reported congenital colour vision deficiency. For the reinforced plastics workers, styrene exposure was monitored on a Thursday via personal sampling throughout the 8-hour work shift, and by measurement of urinary MA levels at the end of the shift. Colour discrimination was assessed by the D-15d test at the beginning of the working day, under standardised lighting conditions. For a

subgroup of 39 styrene-exposed workers and matched controls, testing was also performed before, and immediately after, a one-month holiday. Analysis of the possible reversibility of the effects of styrene in these workers was described by Gobba and Cavalleri (2000), as summarised below.

The geometric mean for airborne styrene concentration was 16 ppm (range 0.8-131 ppm), and end-of-shift MA concentration was 343 mg/l (range 15-3002). With the D-15d test, some styrene-exposed workers and controls made errors along the tritan axis of colour confusion; however "a few" styrene-exposed workers made errors along the red-green axis as well. The control workers in this study were on average older than the styrene-exposed workers, and to correct for the effect of age on colour discrimination, subjects were arranged into 41 pairs matched for age to the nearest two years. In the 41 styrene-exposed workers the mean CCI was 1.26 (SD 0.22), significantly higher ($P<0.01$) than the mean CCI of 1.15 (SD 0.14) for their age-matched controls. Although not commented on by the authors, it appears from the distribution of CCI scores in the 41 styrene-exposed workers that there were two sub-populations; most exposed workers (26/41) had a CCI within one SD of the control mean, but a minority (9/41) had a CCI more than 2½ SDs greater than the control mean. In another method of analysis, all the subjects were arranged into three age groups (16-30, 30-39 and 40-64 years old). Analysis of these three groups revealed that styrene-exposed workers had higher median CCI values than controls, but the difference was only statistically significant for workers of 40 or over. In a further analysis, all exposed workers were subdivided into those with exposures above or below 50 ppm. The results showed that the 15 workers exposed to >50 ppm had significantly greater ($P<0.05$) CCI values than the 55 workers exposed < 50 ppm, although there was no information on worker age in the two sub-groups and a high degree of overlap of individual scores between the two sub-groups of workers. In the low exposure group the median CCI was 1.08, similar to the control median, while in the high exposure group the median CCI was 1.30 with a 90^{th} percentile of 1.64. Although this implies an impairment of colour discrimination, since there is no information on worker age it is not possible to draw firm conclusions about the role of styrene.

For the sub-set of 39 exposed workers who had a mean age of 35 years (SD 6), mean CCI tested before the holiday was 1.23 (SD 0.19); this is on the 95^{th} percentile of the age-related reference value. After the holiday, mean CCI was 1.20 (SD 0.21), suggesting that a 4-week exposure-free period did not influence colour discrimination. However, confidence in such a conclusion is weakened by the fact that there was no testing of control subjects after the holiday, which represented a limitation in the study design.

In the second Italian study, similar methods were used to assess colour discrimination in a group of 40 styrene exposed reinforced plastics workers from 3 factories not included in the previous study (Gobba & Cavalleri, 1993). A control group comprised of 40 subjects, matched for age, alcohol consumption and tobacco smoking, but the population from which they were recruited was not described. Subjects with alcohol consumption greater than 350 g/week or with self-reported congenital colour vision deficiency were excluded from this study population. For the reinforced plastics workers, styrene exposure was monitored on a Thursday by personal sampling, presumably throughout the 8-hour work shift, and by measurement of end-of-shift urinary styrene levels; the frequency of monitoring was not stated. Colour discrimination was assessed by the D-15d test at the beginning of the working day, under standardised lighting conditions.

Among the exposed workers, mean styrene exposure during the 8-hour shift was again 16 ppm. The mean CCI in styrene-exposed workers (1.21, SD 0.20) was significantly higher than the mean in the controls (1.05, SD 0.07). There was no attempt at exposure-response analysis in these workers.

The influence of changes in styrene exposure over a twelve-month period on colour discrimination was assessed in a group of 30 workers (Gobba & Cavalleri, 2000). An

unexposed control group of equal numbers, matched for sex, age, alcohol consumption and smoking, was included. Mean CCI of all styrene-exposed workers at the start of the 12-month period (1.24, SD 0.21) was significantly higher than the controls (1.14, SD 0.14). Among the exposed workers there were 10 individuals for whom exposure increased (Group 1), and 20 individuals for whom exposure was unchanged or decreased slightly (Group 2). The airborne concentration of styrene was monitored by personal sampling and colour discrimination was assessed using the same methods as in the previous two studies.

In group 2 the geometric mean airborne styrene concentration was 14 ppm at the initial time point and 10 ppm one year later; over this time period mean CCI did not change significantly from the initial value of 1.27 (SD 0.18). In group 1 the geometric mean airborne styrene concentration increased over the one-year period from 11 to 16 ppm; in these workers mean CCI increased from 1.18 (SD 0.16) to 1.29 (SD 0.21). None of the mean changes in styrene exposure or CCI over this 12-month period achieved statistical significance. Thus, the reported changes in styrene exposure were small and unlikely to be of any consequence, consistent with the absence of any statistically significant changes in colour discrimination.

Overall, these three Italian studies are consistent in pointing to an association between occupational styrene exposure and impaired tritan colour discrimination. An exposure-response analysis was only carried out in the first study, and this suggested an impairment of colour discrimination in workers exposed to >50 ppm styrene, whereas in workers exposed to <50 ppm styrene colour discrimination was normal. In terms of the magnitude of the effect, the median CCI in the high exposure group (>50 ppm) was 1.30. The results from a sub-group of workers suggest that the effects of styrene on colour discrimination persist even after a 4-week exposure free period. This is in contrast to the Triebig *et al* (2001) study, which showed a clear reversibility of effect; that study is considered to be of superior quality for a number of methodological reasons, including the fact that control subjects were tested after the exposure-free period.

Japanese studies

Three studies in Japanese workers attempted to establish whether styrene has a threshold for effects on colour discrimination.

Eguchi *et al* (1995) investigated colour discrimination in 64 styrene-exposed workers from 6 fibreglass reinforced plastics (FRP) factories and 69 controls recruited from the same and other factories. Exclusion criteria included an alcohol consumption of more than 250 g/week, the presence of congenital colour vision deficiency or poor visual acuity. The mean age of the exposed workers was 38 years (range 18-66) and duration of exposure was 7 years (range 0.2-26.8). The mean age of controls was 38 years (range 20-61). Styrene was said to be by far the most widely-used solvent in the factories; acetone was also used to clean tools. End-of-shift urinary MA concentrations were measured on the same day of colour discrimination testing, but levels were not related to creatinine concentration or ionic strength, although the subjects drank a glass of water 2 hours before urine collection to minimise the effects of differences in ionic strength. Airborne styrene concentrations were measured by area sampling. Colour discrimination was tested using the D-15d test at the beginning of the working day (usually on a Monday) to minimise the possible acute influence of styrene on colour discrimination.

The mean urinary MA concentration in styrene-exposed workers was 220 mg/l (SD 480), and the mean airborne styrene concentration was 21 (range 6.6-36.4) ppm. In both exposed and control groups a number of subjects made errors in colour discrimination. All subjects who made errors made at least one tritan error, with no subjects making exclusively red-green errors. Paired analysis was used to compare colour discrimination in 57 age-matched pairs of styrene-exposed workers and controls. The mean CCI for the 57 styrene-exposed workers was 1.22 (SD 0.23), significantly higher than in the controls (mean 1.12, SD 0.13).

The styrene-exposed workers were also divided into high and low-exposure subgroups according to whether the urinary MA concentration was above or below 420 mg/l. In the low-exposure subgroup of 40 workers the mean urinary MA level was 200 mg/l (SD 110) and the mean CCI was not significantly different from control. However, for the high-exposure subgroup of 17 workers where the mean urinary MA level was 1060 mg/l (SD 930), the mean CCI was 1.33, significantly higher than in controls and low-exposure subgroup. Stepwise regression analysis confirmed a positive correlation between CCI and MA levels, but did not show a correlation with the duration of styrene exposure. It was noted that colour discrimination was not related to alcohol consumption.

The airborne concentrations of styrene in this study related to area sampling, and so are of little use for the determination of thresholds or exposure-response relationships. The urinary MA levels were not corrected for creatinine, and given that workers were encouraged to drink water prior to urine sampling the consequent dilution effect on urinary MA levels means that any extrapolations to estimated airborne concentrations of styrene would be of uncertain reliability. In summary, the results of this study are consistent with an exposure-response relationship for an effect of styrene on colour discrimination, and point to a threshold somewhere between the high and low exposure groups. However, from the data available the exposure levels in terms of airborne styrene concentration cannot be reliably estimated.

The same team studied the exposure-response relationship between styrene and impairment of colour discrimination in a second study using the same methods and exclusion criteria (Kishi *et al*, 2001). Eighty-seven workers exposed to styrene in 7 FRP factories, with mean age 37.7 years (SD 13) and mean duration of exposure 6.2 years (SD 6.2), were tested. They were arranged into low-, medium- and high-exposure sub-groups on the basis of whether end-of-shift urinary MA concentrations were below 100 mg/l, between 100 and 200 mg/l, or above 200 mg/l, respectively. For each subgroup of exposed workers colour discrimination was compared with a group of age-matched controls drawn from 117 subjects from the same and other factories but not exposed to industrial solvents. For the 21 members of the low-exposure subgroup the mean urinary MA concentration was 50 mg/l (SD 30) and the mean CCI was 1.21 (SD 0.26), not significantly different from controls. The 24 medium-exposure workers and 42 high-exposure workers had mean urinary MA concentrations of 140 mg/l (SD 30) and 650 mg/l (SD 700), respectively; for these workers mean CCI values were 1.23 (SD 0.20) and 1.27 (SD 0.27), respectively, both significantly greater than their corresponding control group values.

Again, these results are consistent with the existence of an exposure-response relationship for an effect of styrene on colour discrimination. However, because the urinary MA levels were not corrected for creatinine, it is not possible to reliably estimate exposure in terms of airborne styrene concentrations for the low, medium and high exposure groups.

A recent study by the same team investigated colour discrimination in a group of 57 styrene-exposed workers at a FRP boat plant (Gong *et al*, 2002). The mean age of the exposed workers was 29 years (SD 4), and they had been working with styrene for a mean time of 6.4 years (SD 2.1). Colour discrimination testing was also carried out in a group of 69 non-exposed controls. The mean age of the controls was 38 years (SD 11). Exclusion criteria included congenital colour vision deficiency, the presence of medical conditions that may affect colour discrimination, alcohol consumption greater that 250 g/week, and occupational exposure to styrene for less than 6 months. Styrene exposure was assessed by personal sampling and by measuring urinary concentrations of MA and PGA; in this study these were corrected for the concentration of creatinine in the urine. Both contemporary and historical urinary measurements were available. A cumulative exposure index was calculated for each worker based on frequency and duration of exposure and the results of the monitoring of urinary MA concentrations. Colour discrimination was measured by the D-15d test, conducted immediately before the start of a workshift under standard lighting conditions.

The mean urinary MA concentration at the time of the study was 260 mg/g creatinine (SD 350); this was less than a third of the mean value recorded for 1993. The mean 8 h TWA styrene concentration at the start of the working week was 50 ppm (SD 36). Workers were also exposed to acetone, with a mean 8 h TWA concentration of 49 ppm (SD 25). The mean airborne concentrations of other solvents monitored were low, all less than 0.1 ppm.

CCI was compared in 43 pairs of age-matched subjects, and was significantly higher in the exposed workers than in the unexposed controls; the median values for these two groups were 1.13 and 1.04, respectively. Twenty-three control subjects made no errors (CCI=1.00), compared with 11 of the exposed workers. As in the previous studies, styrene-exposed workers were divided into subgroups. The criterion for grouping was whether the sum of contemporary urinary MA+PGA concentrations was above or below 240 mg/g creatinine. Workers were matched for age, providing 29 high-exposure workers, 29 low-exposure workers, and 29 control subjects for comparison. As found in the previous studies, CCI related to contemporary styrene exposure. For the high- and low-exposure workers, mean CCI values were 1.14 (SD 0.24) and 1.09 (SD 0.13), respectively, both statistically significantly higher than the control CCI of 1.02 (SD 0.04). However, no relationship between cumulative styrene exposure and CCI was found. The relationship between individual CCI values and individual maximum styrene exposure recorded during the previous eight years was investigated. There was a positive, but relatively weak, correlation between maximum MA concentration and CCI.

From the urinary MA+PGA cut-off value of 240 mg/g creatinine, it can be estimated that the low and high exposure groups in this study correspond to mean 8 h TWA exposures of <12 and >12 ppm respectively. However, it is worth noting that this estimate of exposure is based on urinary metabolites measured at the end of a work shift, whereas visual testing was conducted pre-shift generally on a Monday. The strength of evidence for an exposure-response relationship might have been increased if visual testing had also been conducted post-shift on the same day as the urine sampling. It also needs to be noted that the results of this study imply a relationship between contemporary levels of urinary styrene metabolites and an effect on colour discrimination, but no relationship with long-term cumulative exposure was found.

To summarise the 3 Japanese studies, all three consistently point to styrene having a threshold effect on colour discrimination. The Gong study, which had the most rigorous exposure assessment, indicates that the threshold exposure level must be somewhat in excess of 12 ppm (8 h TWA). In relation to severity of effect, all 3 studies conducted the colour discrimination tests pre-shift on a Monday morning, specifically to exclude an acute effect of styrene. This raises the possibility that effects would have been more severe if the tests had been conducted post-shift. Comparing pre- and post-shift results would also have allowed the biological credibility of the results to be more effectively judged, and hence these studies are not thought to be as rigorous as the Triebig study.

Canadian studies

In a series of three reports, colour discrimination was investigated in styrene-exposed workers at 3 Canadian reinforced plastics plants. Unexposed control groups were not included in any of the studies (the study design of these investigations are described in more detail in the “neurobehavioural studies” section).

The initial investigation was based on 81 workers from the 3 plants following exclusions due to self-reported congenital colour vision deficiency, other ocular disorders and injuries, poor visual acuity, or less than 6 months solvent exposure (Campagna *et al*, 1995; Campagna *et al*, 1992). The mean age of the workers was 29 years; mean duration of employment at the plants was 5 years and the mean alcohol consumption was 160 g/week.

Styrene exposure was assessed by personal sampling over 4 h of an 8 h shift and by measuring end-of-shift urinary MA concentration. Visual testing was conducted on Saturday morning, 12 hours after the last exposure. Colour discrimination was assessed using the D-15d test, conducted under standardised lighting conditions. Subjects were classed as having colour vision deficiency if the coloured caps were misplaced by at least two positions, even if this occurred for only one eye.

Mean airborne styrene concentration was 48 ppm (SD 60) and mean urinary MA concentration was 480 mg/g creatinine (SD 690). However, about 1/3 of the workers wore respiratory protective equipment (RPE). 35 workers reported acute eye irritation associated with styrene exposure, and some also reported tearing and blurred vision. The incidence of these symptoms was found to correlate positively with urinary MA concentration; it is unclear whether or not this adversely affected performance in the colour discrimination test. On the basis of the D-15d test, 25 workers (31%) were classified as having colour vision deficiency. Most of these workers made errors of the tritan type, although one made only errors of the red-green type and two made both types of error. Six other workers made errors with no particular pattern. When workers were dichotomised into those with or without colour vision deficiency, statistical analysis showed no significant relationship with urinary MA. The mean uncorrected CCI score for all workers (based on the mean of scores from left and right eye separately) was 1.14 (SD 0.16). Multiple regression analysis was used to investigate the relationships between CCI and age, length of service in the factory, alcohol consumption, respirator use and urinary MA. When CCI was corrected for age, alcohol consumption and length of service, it was found to have a positive correlation with urinary MA concentration but not with airborne styrene levels, presumably reflecting the use of RPE in some workers.

One statistical analysis suggested a positive relationship between CCI and urinary MA, but this was not confirmed by the analysis of those with and without colour vision deficiency. From the mean urinary MA concentration of the workers in this study and using the ACGIH conversion, it is estimated that the mean amount of styrene inhaled by workers is equivalent to an airborne concentration of 30 ppm (8 h TWA). Although there was no control group, it is noted that the mean CCI score from all the exposed workers (1.14) is between the 50^{th} and 90^{th} percentile of normal reference values (Table b), given the mean worker age (29 years). The reports of lacrimation and blurred vision also raise doubts over the interpretation of this study. Overall, there was no consistent evidence for a clear-cut effect of styrene exposure on colour discrimination, and it does not seem possible to draw any firm conclusions from this study.

Follow-up testing on 57 workers from the original study (T_0) was conducted two years later (T_2) (Mergler *et al*, 1996). The mean age of these workers was 32 years (SD 9) and the mean duration of employment at the plant was 6 years (SD 6). Styrene exposure and colour discrimination were assessed as in the previous study, and this report also included the results from a neurobehavioural test battery.

Styrene exposure during the 2-year period, measured as airborne concentration or urinary MA concentration, had slightly decreased in Plant 3, but had broadly stayed the same or showed some slight increases in Plants 1 and 2. It appeared that the reductions in MA between T_0 and T_2 in Plant 3 were largely due to wearing RPE rather than to a reduction in airborne styrene levels. Exposure data for each plant were only presented graphically. The graphs revealed a lognormal distribution of exposure data and that 50% of exposures in each plant were below 50 ppm.

Mean CCI for all 57 workers at T_2 was 1.19 (SD 0.35), similar to the mean CCI (1.18) for these workers at T_0. When the results from each plant were considered separately it was found that in Plant 3 the mean CCI had decreased by 0.20 (SD 0.78). In Plants 1 and 2 where styrene exposure had not decreased, the mean CCI had increased by 0.12 (SD 1.17) although these changes were not statistically significant. When colour discrimination test

results for all 57 workers were grouped according to whether urinary MA had increased, decreased or remained unchanged, there was found to be a statistically significant correlation between urinary MA concentration and increases in CCI over the 2 years. The results of the neurobehavioural test battery showed no decline in any of the 18 test parameters between T_0 and T_2, and a statistically significant improvement in 3 parameters in workers from Plant 3, and an improvement in one parameter in workers from plants 1 and 2.

In summary, the results of the first follow-up showed no decline in colour discrimination in workers where average exposures to styrene had not changed markedly during the two-year interval. There was no information on subjective symptoms of eye irritation in this follow-up study, and the results of the neurobehavioural tests were unremarkable. However, analysis of changes in levels of urinary MA and CCI scores at the level of the individual worker suggests a correlation between increases in the body burden of styrene and deterioration in colour discrimination over the 2 years. It is not possible from the way the results are presented in this study to identify a threshold for an effect of styrene on colour discrimination or to quantify exposure-response relationships. However, qualitatively the results are suggestive of a relationship between exposure to styrene and colour discrimination.

After 9 years, colour discrimination was retested in 18 of the original workers still working at Plant 3 (Castillo *et al*, 2001). The 18 retested workers had a mean age of 38 years and mean duration of employment of 13 years. The authors were unable to enter the plant to sample airborne styrene concentrations or access company records, but end-of-shift urinary MA measurements were made at the end of the working week as in the previous studies. In addition, personal exposure measurements were available for the period 1987 to 1998, and these were combined with individual work histories to produce a cumulative exposure index for each worker.

Urinary MA levels were lower than at year 0 and year 2, and were all below 340 $mg.g^{-1}$ creatinine. Among the retested workers age-corrected CCI had improved significantly between year 0 and year 2, during which time their styrene exposures had also fallen. However, there was no further significant change in CCI between year 2 and year 9, during which time styrene exposure had continued to fall.

In summary, the Canadian studies comprised a series of three cross-sectional studies with ever decreasing size of the study populations at each phase. The lack of an unexposed control group is a possible limitation with these studies. It is difficult to draw reliable conclusions from the first study because of inconsistencies between the different statistical analyses of the results, and uncertainties as to the possible influence of direct eye irritation. It should also be noted that the majority of CCI scores for the workers in this study are likely to have been within the normal age-related range of reference values. The follow-up investigation at 2 years showed no changes in the mean CCI scores over this time period. In some workers within the study population there was a correlation between increases in urinary MA concentration and a decline in CCI scores. Among the small subgroup of workers re-evaluated at 9 years, no changes in mean CCI were detected, although styrene exposure had decreased. These data are suggestive of a possible effect of styrene on colour discrimination, but it is difficult to ascertain the magnitude of any deficiency. Overall, these 3 Canadian studies appear to contribute little to the key questions of this review.

Other studies

The following studies are summarised for completeness, but it is felt that because of methodological problems, or problems in the reporting and analysis of the results, no reliable conclusions can be drawn from these studies.

Chia *et al* (1994) investigated colour discrimination and neurophysiological function in 21 styrene exposed laminators and 21 carpenters from a boat-building plant in Singapore. The

two groups were matched for age, smoking habits and alcohol consumption, although there was apparently no screening for congenital colour vision deficiency. Styrene exposure was assessed by measurement of end-of-shift urinary MA levels. On Monday morning, after an exposure-free weekend, colour discrimination was assessed using the D-15d test under standard lighting conditions, and 10 neurobehavioral parameters were tested. The colour discrimination results were expressed as the geometric mean of the total colour difference score (TCDS).

Mean urinary MA concentrations for the exposed and control groups were 84 and 3 mg/g creatinine respectively. Colour discrimination errors were made by the exposed workers along both the red-green and tritan axes. For the exposed subjects the mean TCDS was 164 (SE 0.4), higher than the mean of 132 (SE 0.4) for the control group. The difference in colour discrimination was highly statistically significant. It was reported that exposed workers performed less well than controls for 3 of the 10 neurobehavioural parameters, but it is uncertain whether or not any useful conclusions can be drawn from this. The TCDS scores for the control subjects, as well as the exposed workers, were exceedingly high relative to age-related normative values. Bowman *et al* (1984) noted that TCDS values in normal healthy subjects of working age are generally below 100. It has been suggested in reviews of the colour vision literature that in this study, the scores had almost certainly been miscalculated (Geller & Hudnell, 1997; Sheedy, 1997). In view of this uncertainty over the results, it is felt that no reliable conclusions can be drawn.

A study of 60 styrene-exposed workers in a French shipyard used the FM-100 test to assess colour discrimination (Fallas *et al*, 1992; follow-up correspondence from Muttray *et al*, 1993; Fallas *et al*, 1993). A group of 60 workers without occupational exposure to chemicals, claimed to be matched for age, intellectual level and ethnic origin, served as controls (the study design is described in more detail in the “neurobehavioiral studies” section). The method of recruiting the exposed and control subjects was not described. Styrene exposure was assessed by area sampling and by determining the end-of-shift urinary MA and PGA concentration. Colour discrimination was tested during working hours, under conditions of daylight, but lighting conditions did not appear to be standardised.

For the styrene workers, mean end-of-shift MA and PGA concentrations were 230 and 57 mg/g creatinine, respectively. The mean airborne styrene concentration over the 3-month study period was 24 ppm, although peaks of up to 469 ppm were measured. How these values relate to personal exposures is uncertain. In the styrene-exposed workers the mean total error score (TES) value was reported to be 260 (SD 137), and was not significantly different from in control workers (mean 263, SD 114). However, 32 out of the 60 exposed workers styrene made circumferential or major errors in the test; this was significantly higher than for the controls (20 out of 60). This difference was due to an increased incidence of both red-green and tritan errors.

One limitation of this study is that there was no attempt to any exclude subjects with congenital colour vision deficiency, so it is possible that bias may have been introduced. Another problem is that the mean colour discrimination error scores reported for the controls are unexpectedly high, since FM-100 TES scores of less than 100 are generally expected in individuals of working age with unimpaired colour discrimination. The high TES values could have been generated if the scores had been miscalculated by the study organisers, as suggested by Sheedy (1997), who speculated that the correct mean TES values could be 90 in exposed workers and 93 in control workers respectively. Either way, the mean TES values for the two groups are similar. Overall, there are doubts about the reporting of the results of this study, and it is felt that no reliable conclusions can be drawn.

A study of workers in a Soviet air-conditioner factory reported that in 153 workers exposed to 2-3 times the national OEL for styrene, the thresholds for colour perception were higher than in 178 control workers at the same factory (Alieva *et al*, 1985). The Rapporteur was able to

access this report only in abstract form, but further information on this study is available in a review by Sheedy (1997), who commented that the methodology for measuring colour discrimination was non-standard and the description of exposure assessment in the original report was inadequate. Consequently, again it is felt that no useful conclusions can be drawn from this report.

Summary of studies on colour vision

The most well reported and conducted studies are those by Triebig *et al* in German workers, and the 3 studies each in Italian and Japanese workers. These studies provide some evidence to support the view that styrene does cause changes in colour discrimination relative to age-matched controls. However, it should be noted that, as a single colour vision test rather than a testing battery approach was used, these findings are not sufficiently robust to reliably characterise the scale and the nature of the effect. Generally, the effect was on the tritan (blue-yellow) type, although some workers also had evidence of red-green colour vision deficiency. However, there is a lack of information on whether the effect is related to short-term or repeated long-term exposures to styrene. There is also a lack of information on the magnitude of the effect in workers exposed to styrene concentrations > 100 ppm (8h TWA), although performance in colour discrimination tests at these high exposures is likely to be subject to confounding because of transient CNS depression and eye irritation. Having concluded that styrene exposure does cause changes in colour discrimination, key issues that need to be resolved are whether a threshold exposure level can be identified and whether the effect is reversible or persistent.

Regarding threshold exposures, the results of the Triebig study suggest that no deficiencies in colour discrimination would be expected in those exposed to less than about 20 ppm styrene (8 h TWA). At all time points in this 2-phase study, effects on colour discrimination were described by the study authors as “mild”, and the styrene-exposed workers were said to have no conscious awareness of any impairment. Of the Italian studies, analysis indicated that workers exposed to >50 ppm styrene had significantly poorer colour discrimination that those exposed to <50 ppm. The latter group had a median CCI value of 1.09, similar to control values. In one of the Japanese studies (Gong *et al*, 2002) workers were placed into a high or low exposure group according to whether urinary MA+PGA levels were above or below 240 mg/g creatinine, corresponding to an airborne styrene concentration of about 12 ppm (8 h TWA). The results suggested that colour discrimination would only be impaired in those exposed to above 12 ppm, but a precise threshold could not be determined. Taking all these results together, it appears that changes in colour discrimination would not be expected with 8 h TWA exposures below 20 ppm.

In relation to reversibility, the Triebig study provides the most rigorous and systematic investigation of this issue, in that in the same workforce colour discrimination was assessed pre-shift at the start of a working week, post-shift on a Thursday, and after a 4-week exposure free period, with a repetition of these same test conditions one year later after a reduction in exposure levels at the plant. In both phases of the study, the results of post-shift testing showed a markedly higher proportion of subjects making test errors in the styrene-exposed group compared to controls. Colour discrimination in exposed workers tended to be worse post-shift on a Thursday than at the start of the working week. After a 4-week exposure-free period, there were no differences between styrene-exposed and control workers suggesting that the impairment of colour discrimination was fully reversible. The reproducibility of this pattern of findings after 12 months strengthens the evidence from this study. In contrast, in one of the three Italian studies, colour discrimination was tested in a sub-group of workers before and immediately after a one-month holiday. There was no improvement in mean CCI in this sub-group after the holiday, suggesting a persistence of the effects of styrene. However, the control workers were not tested after a one-month holiday to confirm the stability of the control test scores, and so this study was not as

rigorous as the Triebig study. The Japanese studies are of little help in resolving the issue of reversibility, in that in each of these studies, colour discrimination testing was only conducted pre-shift, specifically to avoid the potential for any acute effects of styrene. In summary the body of data on the issue of reversibility of the effect of styrene is not extensive and is inconclusive. The results from the most rigorous study in which this was investigated point to a reversibility of effect, whereas results from a somewhat less well-conducted study suggest a persistence of effect. Overall, no firm conclusions can be drawn regarding reversibility.

In relation to mechanisms, one possible mechanism by which solvents might reversibly affect colour discrimination is by a transient effect on electrical membrane potentials in the retina. A study in anaesthetised cynomolgus monkeys used an electroretinogram to study the effects of solvents on the electrophysiology of the retina (Skoog & Nilsson, 1981). Within 20 minutes of intravenous administration of styrene and toluene at doses of 3 to 15 mg/kg and 0.3 to 60 mg/kg respectively, there was a hyperpolarisation of the electrical potentials of the retinal membrane and the eye. It has been calculated that these intravenous concentrations will be produced by 20 minutes exposure to airborne concentrations of approximately 50 ppm styrene and 100 ppm toluene respectively. Although such an effect of solvents on signal transduction at the retinal membrane could be responsible for acute effects on colour discrimination, since in human volunteer studies 30 minutes inhalation exposure to 300-350 ppm toluene was found to have no acute effect on colour discrimination (Muttray *et al*, 1999), there is doubt on the relevance of these findings in animals to the mechanism of colour discrimination impairment in humans. Another possible mechanism is by an effect on the content of neuramines in the retina. A recent non-standard study (Vettori *et al*., 2000; see study in animals) in rats exposed by inhalation to 300 ppm styrene (the only concentration tested) for 12 weeks has shown that the content of neuramines (dopamine and DOPAC) and glutathione in the retinal cells of the exposed animals was lower than in controls. However, again, the relevance of these findings in animals to the mechanism of colour discrimination deficiency in humans is unknown, especially if it is considered that rats are dichromatic and humans are trichromatic.

Another issue that needs to be addressed is the health significance of these changes in colour vision observed in styrene-exposed workers. Age-related reference CCI values obtained in healthy individuals with the D-15d test have been reported in the literature; the reference values of Muttray et al (1998a) have been reproduced in Table (b).

Table (b). Distribution of age-related CCI scores with the D-15d test in a population of healthy workers (taken from Muttray *et al*, 1998a)

Age No of workers	19-25 (n=57)		26-35 (n=93)		36-45 (n=73)		46-55 (n=56)		56-62 (n=17)	
Eye	1st eye	2nd eye	1st eye	2nd eye	1st eye	2nd eye	1st eye	2nd eye	1st eye	2nd eye
50th percentile	1.0	1.0	1.0	1.0	1.06	1.0	1.12	1.07	1.20	1.17
90th percentile	1.18	1.16	1.22	1.12	1.19	1.18	1.41	1.31	1.72	1.41
95th percentile	1.22	1.25	1.29	1.21	1.25	1.22	1.55	1.34	1.72	1.61

Some investigators have used the 95th percentile of these CCI reference as a criterion for judging “abnormal” colour discrimination. Thus, CCI scores that are markedly above the

normal age-related reference values can be judged as abnormal, and can give some indication of the severity of effect. Moreover, one way of judging the functional significance of any CCI difference is to relate it to the changes that normally occur as a result of ageing (Sheedy, 1997). For example, a comparison of CCI values reported for the D-15d test in the Triebig study (2001) with the age-related norms in Table (b) indicates that the styrene-exposed workers, who had a mean age of 38, had CCI values in the range expected for individuals aged about 60.

One possible criterion for assessing the health significance of this effect could be whether or not the changes are severe enough to affect a worker's ability to carry out a particular job, such as colour matching in paint manufacturing or printing. Information on this aspect is available from a recent study (Muttray *et al*, 1998b). The ability of a group of rotogravure print workers to mix inks correctly was compared with the results of colour discrimination testing assessed in these workers by a battery of tests including an anomaloscope and the D-15d and Ishihara tests under standard illumination. The ability to mix red, yellow and blue inks to a required composition was assessed by the senior staff member who was known to have perfect colour discrimination. The workforce consisted of 38 printers and print preparation staff and had a mean age of 41 years (range 19 to 66). Of these 38 workers 5 were found to have congenital colour vision deficiency, and had difficulties matching colour. One of these workers was already aware of his condition, and in the past had sought help when colour matching. None of the remaining 33 workers without congenital colour vision deficiency had difficulties matching colour. Of these workers 17 had perfect scores with the D-15d test (CCI = 1), 5 made only minor errors and 11 who were found to have abnormally high CCIs, due to tritan errors. For this last group mean CCI values were in the range 1.05 to 1.29. This study suggests that workers making errors with the D-15d test of the magnitude reported among styrene exposed workers may well be able to perform jobs that require good colour discrimination.

Another criterion for assessing the health significance of this effect could be whether or not the worker is consciously aware of it. Information on such a factor can be obtained from one of the studies available. Triebig *et al* (2001) reported that styrene-exposed workers making errors in colour discrimination tests were not aware of any deficit.

The issue of reversibility will also influence views on the health significance of styrene-induced colour discrimination changes. The most rigorous study (Triebig *et al*, 2001) suggests that effects on colour discrimination are fully reversible within weeks following cessation of exposure, and showed significant improvement after an exposure-free weekend.

Overall, although there are findings suggesting changes in colour discrimination at relatively high levels of exposure to styrene (≥ 50 ppm, 8h TWA), it should be noted that the available studies are not sufficiently robust to reliably characterise the scale and nature of the effect. Also, given the very mild nature (the affected individuals were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, it is deemed that the slight changes in colour discrimination detected should not be considered as an adverse health outcome of styrene exposure. It can be concluded that, since the effects observed at 50 ppm (216.5 mg/m^3; 8h TWA) are not yet adverse, this exposure value can be considered a NOAEC.

Biochemical studies related to nervous system functioning

This is an area of research which is at a very early stage in its development and is therefore not using standard methods. There is no recognised framework available for the interpretation of the results. In some cases there is no established basis, such as a known interaction between solvents and a given enzyme, for investigating the parameters chosen

by the authors. Potential neurotoxicity markers were investigated in 60 styrene-exposed workers (44 men, 16 women) from three plants in the USA (Checkoway *et al,* 1992). The neurotransmitter-related markers analysed have no known clinical significance in themselves but reflect biochemical processes in peripheral blood cells which are also known to occur in the brain. The implication is that, if changes are found in peripheral blood cells, these may indicate that styrene has the potential to modify neural function in the brain, via the same underlying mechanism. However, such an extrapolation has not been firmly established. The percentage of the total workforce participating in the study was not reported. A group of 18 non-exposed workers (14 men, 4 women) from one of the 3 plants in the study were used as controls. Medical histories, neurological symptoms and alcohol and smoking histories were obtained by questionnaire. Blood samples were analysed for sigma non-dopaminergic, non-opioid receptor binding in lymphocytes, monoamine oxidase type B activity (MAO-B) (catalyses oxidation of dopamine) and serotonin uptake in platelets. The ability of certain anti-psychotic drugs to bind to the sigma receptor was measured; there is no known physiological function of the sigma receptor in lymphocytes. Blood styrene concentrations were used to form three groups of workers, those with <0.05, 0.05-0.19, and 0.20 or above mg styrene/ml. Differences between the groups in factors such as age and alcohol consumption were evaluated by covariance adjustments when the results were analysed. there was evidence that styrene exposed produced CNS-related symptoms in the exposed workers. The prevalence of “headache” for example was 39% in non-exposed and 60% in the highest-exposed group. Similarly “light headedness” was reported by 17% of non-exposed and 60% of the highest-exposed group. There was no difference between the groups in sigma receptor binding. The uptake of serotonin by platelets increased with increasing blood styrene level; the trend had a “weak increasing gradient”. MAO-B activity decreased with increasing blood styrene level and with the number of symptoms reported. However, in the more detailed statistical analysis, relatively little of the variance of MAO-B activity could be explained by exposure to styrene. Overall, there is no legitimate scientific basis from which to connect the results, via an established biological mechanism, to styrene exposure, nor to attribute toxicological significance to the findings.

Enzyme activities and prolactin levels linked to dopamine activity were assessed in samples taken from workers exposed to styrene (Bergamaschi *et al,* 1996). The authors had decided to test the hypothesis that organic solvents could produce changes in catecholamine metabolism, which in turn could lead to the effect on the nervous system. Monoamine oxidase type B (MAO-B) activity measured in platelets (suggested by the authors to be a surrogate for MAO-B activity in the nervous system), serum dopamine β-hydroxylase (DBH) (catalyses formation of noradrenaline from dopamine and is released from adrenal medulla and peripheral and central neurons) and serum prolactin (PRL) (dopamine inhibits prolactin secretion) were investigated. The test group comprised 53 workers exposed to styrene due to their employment in GRP factories, with a non-exposed group of 60 local blue-collar workers from industries where “occupational risk factors were ruled out” being used for comparison. Details of recruitment and selection were omitted from the report. In the exposed group, the mean age was 37.8 years and the workers had been employed in GRP work 9.3 years on average. The control group was comparable with respect to sex, age, and other recognised potential confounders, including medical histories. The sum of mandelic and phenylglyoxylic acid metabolites was obtained from urine samples collected 15 hours after the last exposure. “Ambient monitoring” indicated that styrene concentrations were in the range 5-100 ppm (8-hour TWA). Blood samples were taken before the start of a working shift (at approximately the same time as the urine samples were collected). Relative to the controls, DBH was statistically significantly lower, PRL statistically significantly higher and MAO-B was slightly lower (not statistically significant) in the exposed group. DBH activity remained significantly correlated (in a negative sense) with metabolite levels, after adjustment for alcohol consumption. Styrene exposure variables did not correlate with MAO-B activity. Two subgroups were identified on the basis of metabolite measurements, with median levels of 89 and 340 mg/g creatinine. Relative to controls, DBH activity was lower in

both subgroups; MAO-B was decreased only in the higher metabolite group. The prevalence of abnormally low DBH activity values, defined as "<5% control values" without further explanation, was determined. Abnormally low DBH values were found in 17% of the low metabolite group and 42% of those in the high metabolite group using this definition. Similar results were seen in relation to abnormally high PRL values.

However, in a similar study by the same authors (Bergamaschi *et al,* 1997), there was no significant difference in DBH activity between styrene-exposed and control groups. This is in contrast to the findings above in the 1996 study. It was noted that the most exposed workers had significantly lower DBH activity than the average control level. MAO-B activity was also not statistically significantly altered in the styrene-exposed workers, but prolactin values were significantly higher than controls. Of the 3 parameters, only DBH was significantly related to levels of urinary metabolites of styrene. Hence, the results of the two studies of Bergamaschi *et al,* taken together, are rather inconsistent and (taking into account MAO-B results), the inconsistency increases when the above study of Checkoway *et al* (1992) is considered.

More recently, Luderer *et al*. (2004) investigated whether the effect of styrene exposure on PRL secretion was an acute or chronic effect. Styrene-exposed workers (254 men and 43 women) were recruited from 17 workplaces in the reinforced plastics industry. Personal breathing zone air styrene, whole blood styrene and serum PRL were measured during 1 to 3 sessions, approximately 1 year apart. Linear multiple regression was used to model the relations between acute (air styrene or blood styrene obtained at the same time as the PRL levels), subchronic (average air or blood styrne over 2 or 3 sessions) and chronic (years of work X average air styrene over 2 or 3 sessions) indices of styrene exposure and serum PRL levels.

Air styrene concentrations ranged from <1 to 142 ppm, with a median of 9 ppm (10.6% of the samples were below the limit of detection). Blood styrene concentrations ranged from <0.001 to 2.05 mg/l with a median of 0.0089 mg/l (7% of the samples were below the limit of detection). Blood and air styrene concentrations were highly correlated with one another ($r=0.77$, $p<0.001$). Acute blood styrene concentration was the strongest predictor of serum PRL concentration, with the model predicting a 2.06-fold increase in PRL levels for every 10-fold increase in blood styrene. Serum PRL levels tended to increase with increasing styrene exposure in both men and women; however, women tended to have higher PRL levels. Overall, the results from this study confirm that styrene exposure (median level of 9 ppm) enhances serum PRL concentrations and support an acute effect of styrene on PRL secretion.

There are indications of lower levels of serum DBH activity and higher levels of serum PRL with styrene exposure (mean levels between 25 and 58 ppm). There are also indications that increased PRL secretion is an acute rather than a chronic effect of styrene exposure. However, the evidence is not robust, an underlying mechanism not clearly established, and the clinical consequences not characterised. Furthermore, the importance of these results in the understanding of neurotoxicity has yet to be established; also in comparing exposed to a non-exposed comparison group the problems of unmeasured confounding is substantial because the relevant confounders are unknown. Overall, it is suggested that these findings should not be used in characterising the toxicological hazard assessment of styrene.

Neurobehavioural studies

Neurobehavioral testing was developed, and can be used to follow or define the changes in CNS function experienced by a subject in whom the presence of a CNS abnormality has already been diagnosed by other means (e.g. a head injury case). However, where neurobehavioural test results are collected and reported in isolation, deficits in performance

do not necessarily imply a clinical neurological disease. Unfortunately, in this latter situation, there is as yet no interpretational framework that has been widely recognised and agreed. This is the case for most of the data available in relation to styrene exposure. In almost every study in this section, workers were recently exposed to styrene prior to testing; any neurobehavioural changes reported could have been a reflection of an acute effect of recent exposure or the consequences of long-term exposure. Volunteer studies, reported in the single-exposure section, indicate that there is no convincing evidence of reduction in performance in neurobehavioural tests with exposure at 100 ppm for 7 hours. A slight decrease in performance was observed at 200 ppm for 90 minutes and 350 ppm for 30 minutes.

A neurobehavioural testing battery (Neurobehavioural Core Test Battery) was used to assess performance of GRP workers exposed to styrene in Canada (Mergler *et al,* 1992). In addition, a questionnaire on health symptoms (profile of mood states) and an olfactory perception threshold test were administered. From a total of 151 workers employed in 3 plants, 136 (91%) volunteers were recruited for the exposure assessment part of the study. Of the 128 (85.6%) workers that agreed to participate in the performance and function testing, 11 were excluded for reasons which included less than 6 months work at the plant, history of exposure to neurotoxic substances and head injury. Each participant was questioned about work history, smoking habits and alcohol consumption. The test battery included aiming test (motor stability), simple reaction time, Santa Ana (manual dexterity), H cancellation (attention and perceptual motor speed), Wechler memory scale and Benton visual retention test (visual memory). Testing and questionnaires were administered 12 hours after the last exposure. The exposure assessment results were reported separately (Truchon *et al,* 1992). Personal air sampling data were gathered throughout a shift and post-shift urinary mandelic acid measurements obtained for each worker. The highest 8-hour TWA arithmetic mean exposure levels of 131, 120 and 117 ppm were found in the chopper-gun operator (moulding), painters and laminators groups respectively. The same groups had mandelic acid levels of 0.73, 0.56, 1.26 mmol/mmol creatinine. The workers were divided into two groups according to urinary post-shift mandelic acid (>0.6 or <0.6 mmol/mmol creatinine). The groups were similar in age, level of education and alcohol consumption but the higher metabolite level group had been employed for longer. Fluctuation in mood (41% vs 9%) and symptoms of fatigue were reported at a higher rate in the higher exposure group. Performance testing results were adjusted for age, education and alcohol consumption. There were no statistically significant differences in performance in the neuobehavioural tests, although lower average results were recorded in the aiming test, memory scale and H cancellation test in the higher exposed group. Olfactory function was significantly decreased in the higher mandelic acid level group but detailed results were not reported.

The authors of the study described above conducted a re-assessment of the workers in a follow-up study using the same procedures (Mergler *et al,* 1996). The manufacturing plants had taken steps to reduce exposure of the workers to styrene following the first study. Two years after the first study, workers were re-examined and exposure measurements obtained as before. Of the 118 workers with complete data in the first study, 75 were still employed and 57 (76%) agreed to be re-tested. This group available for the second study was of longer employment and higher exposure than the group from which they derived. However, no information (e.g scores and levels of mandelic acid obtained during the first study) was provided on those who had left the plant. Improvement (lowering) of exposure was only observed in one of the 3 plants. When the results of all testing were compared for the two time points, significant differences were found in performance in the Santa Ana, digit span and digit symbol test between the administration of the tests in the first and second studies. (Two of these tests were not listed in the methods used in Mergler *et al,* (1992) but would be expected to be in the core test battery). The performance on the other tests altered in both directions, i.e. better and worse performances were observed. The authors indicate that they consider that factors other than changes in the level of styrene exposure, such as

differences in test administration and conditions of testing, could account for these differences. When the 57 workers were grouped according to changes in their mandelic acid levels (increased, the same or decreased) over the two years a significant relation was found with simple reaction time, digit symbol forward and tension and fatigue on the mood scale, indicating that those whose mandelic acid levels had increased performed worse. However, no improvement was seen in those whose mandelic acid levels had decreased. Also, in view of the large number of tests carried out, it is unclear which of the positive responses may have been due to chance.

A recent neurobehavioural study was conducted with 30 GRP laminating dockyard workers in France (Jegaden *et al,* 1993). The total number of exposed workers at the dockyard was not stated and full details of the selection were not apparent. Controls (30) were taken from other types of work and matched for age, sex, ethnic group, intellectual and socio-economic level. Any potential participants with likely confounding disorders or exposures were excluded. Exposed workers had a history of 5 years on average working with styrene. Simple reaction time, vigilance reaction time and digit span short-term memory (Wechsler memory scale) tests were administered both early on Monday morning (i.e. after 2 exposure-free days) and immediately after Monday work shift. Mean personal exposure concentration was 23 ppm (range 4-55 ppm) (8-hour TWA). In both control and exposed groups, results were improved at the end of the working day relative to pre-shift scores; this could be an effect of practice causing improvement on the test. No single-exposure effect of styrene was detected. Statistically longer reaction times and statistically poorer memory results were measured in the exposed group when compared to controls in both the morning and evening. The exposed group had reaction times of 290 ms in both the morning and afternoon; values for the control group were 270 and 250 ms. However, there was no significant difference between exposure subgroups of 1 year or 8-14 years exposure; a correlation with duration of exposure might have been expected if styrene was the causative agent. Therefore there was no clear evidence for an effect of repeated exposure to styrene in this study.

Neurobehavioural tests were administered to a group of 60 male workers exposed to styrene while building GRP ships in France (Fallas *et al,* 1992). The total number in the exposed group and the selection procedure for the study group were not given. The mean exposure level was 24 ppm in the workplace (apparently measured continuously during 3 months with the highest peak being 469 ppm). The mean duration of exposure was 6.5 years. A control group of 60 people was matched for age, intellectual level and ethnic origin. Smoking habits were similar in exposed and control groups. In the exposed group there was no significant exposure to other chemicals and no histories of alcoholism or disease likely to affect the outcome of the tests. Psychometric WHO core battery tests were used. These were simple reaction time, digit span (memory), Santa Ana (manual dexterity), digit symbol (perceptual motor speed), Benton (visual perception/memory) and aiming (motor steadiness/speed) tests. Assessment of mood (Profile of mood states, POMS) and medical history questionnaires were included in the study. The day of the week chosen for the tests was not specified. No overall significant difference or exposure trend was found for the POMS questionnaire. There was a statistically significant difference between the groups only in the results of the motor steadiness/speed test and in a subgroup exposed to styrene for >10 years in the memory test. From the few data reported it was evident that some workers were exposed to styrene levels above 300 ppm in this study; single exposure effects have been observed at these concentrations in volunteers and may have contributed to any findings in this study.

A neurobehavioural testing battery (Neurobehavioural Evaluation System, NES) was used to assess performance of GRP workers exposed to styrene in the US (Letz *et al,* 1990). This study was designed to examine acute effects of styrene exposure. Three tests from NES – continuous performance test (sustained visual attention), hand-eye coordination test (manual

dexterity and coordination) and the symbol-digit substitution test (perceptual motor speed) – were administered to 105 participating workers (some of whom were exposed to styrene) pre-shift, midday and post-shift. All exposed workers from the 5 companies in New England which were willing to enter the study were asked to participate. There had been a few exclusions on the grounds of features such as previous head trauma. The mean age was 33 years and mean duration of styrene exposure 4.6 years. The day of the week chosen for the testing was not reported. Personal monitoring results obtained during the period of testing on exposed workers were 8-hour TWA geometric mean 13.5 ppm. There were 23% 8-hour TWA samples >50 ppm and 8%>100 ppm. There was better performance on all tests at midday and post-shift possibly due to a learning effect on repeating the test. Performance in the tests was related to age and level of education but not to years of exposure or self-reported alcohol consumption. Results of the symbol-digit substitution test conducted in the afternoon were statistically significantly worse with increasing exposure (using personal air sample results) when comparing exposure groups after adjustment for age and education level using regression analysis. There was also a significant difference in symbol-digit performance in the afternoon comparing <50 ppm and >50 ppm as a dichotomous variable. Performance was lower in the higher exposure group and the authors considered that exposure levels above 50 ppm accounted for the majority of the effect of styrene exposure apparent in the study overall. No effect of styrene exposure was found for the other tests. Duration of exposure (years in the job) was not related to test performance. Since only the after-shift results were analysed the effect measured in the study could have been related to exposure to styrene during the shift and not necessarily long-term exposure.

In another study, 50 exposed subjects from the GRP industry and 50 controls in manual work in the same region of Italy were selected (Mutti *et al,* 1984). Exclusion criteria included diseases such as diabetes, renal failure, neurological disorders and high cigarette or alcohol consumption. The control workers were not known to be exposed to neurotoxins. They were matched for age, sex, education level and score on the Wechsler adult intelligence scale vocabulary test. A range of neurobehavioural tests were administered on a Saturday morning, 15 hours after the last exposure to styrene. The test battery consisted of block design (visual memory), digit symbol (perceptual motor speed), word-learning/logical memory tests short-term and 30 minute recall), embedded figures (visual perception) and choice reaction time. It was stated that styrene exposure was 10-300 ppm (mean duration was 8.6 years) but no associated details were given. Urinary metabolite levels were measured in samples collected before tests were conducted (Saturday morning) and the results used to indicate styrene exposure. In the exposed group, the test results were generally statistically significantly poorer than in the control group (apart from digit symbol test). This result might indicate a long-term underlying difference between higher levels of metabolites or duration of exposure and lower scores on block design and choice reaction time, having controlled for age and vocabulary score. The exposed group was subdivided into 4 groups (9-14 subjects) on the basis of metabolite concentrations. Each score was compared with that of the matched control. The difference between the two was then used to analyse effect against this indicator of dose. In block design, logical memory and reaction time tests, there was a dose-response relationship (poorer scores at higher metabolite levels). The other test results did not show a dose-response in this analysis. Due to the inadequate reporting of the exposure conditions it is not possible to determine a clear relationship between airborne exposure level and any effects seen in this study.

Neurobehavioural tests were administered to a group of 86 workers from 6 GRP plants in Taiwan (Tsai and Chen, 1996). Although 177 were initially entered into the study, some refused and some failed to complete the adapted NES-2 battery of neurobehavioural tests and others were excluded for reasons such as alcoholism. Information was gathered on smoking history and other lifestyle factors and subjects were asked to complete subjective symptom, occupational and medical questionnaires. The battery comprised finger tapping, continuous performance, associate learning, symbol-digit, pattern comparison, pattern

memory, visual digit span, switching attention, associate delayed recognition and mood scales. A vocabulary test was also administered. All the tests were administered away from the workplace and at least 16 hours after last exposure. Exposure to styrene (personal and area sampling) was measured on typical work days. Using 8-hour measurements, a non-exposed group of 45 workers (mean 1.0 ppm) and exposed group of 41 workers (mean 21.9 ppm) were identified. Multiple regression analysis was applied "to control for possible confounding factors" of age, years of education, gender and alcohol consumption. Only the continuous performance (reaction performance in response to recognition of a specific visual stimulus) was significantly associated with styrene exposure. However, it is not certain that this result was due to chronic rather than acute effects of styrene exposure. Numbers of reported symptoms were not significantly correlated with styrene exposure.

A styrene exposure index was derived for 98 GRP workers in a study of neurobehavioural performance in Finland (Lindstrom *et al,* 1992). This came from measurements of styrene in air, metabolites in urine, number of years exposed and number of hours laminating in the job description. Mean job duration was 12.7 years. The mean styrene concentration in air near the workers was 30 ppm (reference period and range not stated). There was no control group. Subjects had been excluded if they had a history of potentially confounding factors such as diabetes or head injury. Each worker was subjected to tests from the Wechsler Adult Intelligence Scale WAIS) and Wechsler Memory Scale (WMS and a POMS questionnaire was administered. Testing was conducted in the early morning on a day following a normal working day. The tests were verbal assessment, sign test for visuomotor learning and speed, cube test for visuoconstructive reasoning, symmetry drawing (visual perception/memory), Bourdon Wiersma test (perceptual motor speed test), finger taping (simple motor speed), WMS numerical series, retention of learned associations and visual reproduction test. Intelligence level was said by the authors to be a good average level for the national population of the same age but education level was lower in a laminators subgroup of the total workforce. Age but not styrene levels in air or metabolites in urine were seen to correlate with test performance. A variable describing alcohol consumption was not related to test performance. The overall exposure index was related to slightly impaired performance in the Bourdon Wiersma test and to symmetry drawing reversions and forgetfulness in questionnaire results. When education level was adjusted for, correlation between results of the same two neurobehavioural tests and WMS numerical series test and laminating hours and between symmetry drawing reversions and exposure index were statistically significant. Since the workers were exposed on the day before the tests, any effect could be accounted for by exposure to styrene in the previous shift.

The results of a detailed study of psychological/psychomotor function in a group of 98 workers exposed to styrene at various Finnish factories making polyester plastic products have been reported (Härkönen, 1977 and Lindstrom *et al,* 1976). The incidence of symptoms of toxicity (fatigue, impaired concentration, headaches and dizziness) and the results of neurophysiological tests (electroncencephalography and peripheral nerve conduction velocity) have been summarised under the "Neurological surveys in workers occupationally exposed to styrene" section of this review (Seppalainen and Härkönen, 1976; Härkönen *et al*, 1977; Härkönen *et al,* 1978a and 1978b). Urinary mandelic acid levels were monitored in these workers at the end of the work-shift over a five-week period. The mean age of the workers was 30 years and the average duration of exposure five years. Each individual was subjected to a battery of tests from the WAIS Wechsler adult intelligence and memory scales; reaction time (visuomotor speed), Santa Ana test (manual dexterity), Kuhnburg figure matching test (visuomotor speed/memory), Bourdon Wiersma test (perceptual motor speed), symmetrical drawing test (visual perception/memory), and Mira test (motor steadiness). In addition personality tests (Rorschach ink blot test) were also included. None of the workers studied had diabetes, epilepsy, neurological disease, or any history of unconsciousness for more than 30 minutes; details of any drug used were known. The results in these workers were compared with those obtained using a control group of 43 concrete reinforcement

workers of similar age (33.5 years). These men were stated to be of comparable education level, and from similar geographic location to the styrene-exposed men but this might not indicate good matching. None had any history of neurological disease or unconsciousness.

For the majority of measurements made there were no significant differences between the two groups. Small but statistically significant differences were noted in two tests only. In the exposed group visuomotor speed/perception was poorer in one test, and latency was longer in the Rorschach test. Somewhat more marked differences but again limited to two tests only were noted where the results obtained in 19 styrene workers with post-shift urinary mandelic acid levels above 1762 mg/l. Significantly poorer results were obtained in the workers with higher mandelic acid levels in tests for visuomotor speed and perception (the Bourdon Wiersma and the symmetry drawing tests), and the Mira test for motor steadiness. It was suggested, on the basis of previous studies carried out at the Institute, that these urinary mandelic acid levels were equivalent to an 8-hour TWA exposure to styrene of 75 and 25 ppm respectively but this cannot be considered to be reliable because there is wide variation in kinetics between individuals (see toxicokinetics section). The exposed group was further divided in an attempt to define more closely the lowest mandelic acid concentration at which statistically significant effects occurred. Poor visuomotor perception was noted in the symmetry drawing test in workers with a post-shift urinary mandelic acid concentration of 800 mg/l and above; reduced accuracy was noted in both this and the Bourdon Wiersma test in these exposed workers with mandelic levels of above 1200 mg/l. Impaired motor steadiness, using the Mira test, was also noted in such workers. These results suggest that some slight effect on visuomotor perception and, to a lesser extent in motor steadiness, occurs in workers exposed to styrene. However, it is not possible to reliably relate the results to airborne exposure levels.

Personality results from the Rorschach inkblot test on the same exposed and control groups as above were reported (Lindstrom and Martelin, 1980). There were few emotional reactions, low anxiety and a low number of neurotic signs. Overall there was no clear effect of styrene exposure on personality factors.

Neurobehavioural tests were administered to 23 workers (percentage participating not given) in the GRP industry in Germany (Triebig *et al,* 1989). These were simple and complex reaction times, pre-exposure and actual intelligence, learning, memory and personality change tests. The tests were administered before and after the shift on Monday (ie after an exposure-free weekend). Exclusions had been made on the basis of head injury, alcohol abuse and psychiatric disease. No significant differences were found between pre and post shift results nor when results were compared with 23 controls matched for age, socio-economic status and pre-exposure intelligence level and lacking solvent exposure. Duration of exposure was not given. Mean styrene exposure was 18 ppm, 7-hour TWA, (range 3-251) with peak concentrations of 140-600 ppm during lamination. At one of the four companies the mean 7-hour TWA exposure level was 136 ppm; this indicates that exposure levels varied considerably across the group of 23 workers.

A group of workers exposed to styrene at a UK factory making glass fibre boats has also been subjected to a range of neurobehavioural tests (Cherry *et al,* 1980). Most (27/30) of the workers exposed to styrene at the plant were included in the study. The mean age of the men was 23 years; number of years exposed was not stated. A battery of six tests was used to measure different aspects of performance. These were a simple reaction time test, vigilance test, digit symbol substitution test (perceptual motor speed), forward and backward digit span memory test and an assessment of mood (principally feelings of tiredness and related effects) using a visual analogue scale. Tests were administered on a Monday morning after a weekend free of exposure. The results obtained were compared with those found in a control group of 27 men, of average age 26 years, employed at the factory but not expected to be exposed to styrene. The two groups were fairly similar in age and education

background. The application of exclusion criteria such as past head injury and neurological disease was not discussed. Styrene levels were measured throughout the shift using personal monitors. Mean 7-hour TWA values during the morning and afternoon were 117 and 52 ppm respectively; the overall mean 7-hour TWA exposure throughout the shift was 92 ppm. Blood styrene levels and urinary mandelic acid levels were determined at the end of the shift. Blood levels were in the range of 2-20.9 mmol/l. In addition, blood styrene levels were determined in the control group; small but detectable amounts were present, in the range 0.2-1.2 mmol/l. The exposed workers were divided into two subgroups on the basis of whether blood styrene levels were above or below 5.4 mmol/l. There were 14 workers in the former group and 13 in the latter.

The results of the visual analogue screen for assessing effects on mood and physical or mental tiredness indicated some tendency to deterioration in both exposed and control groups over the work-shift. The effect was not statistically significant. However, a statistically significant effect was noted when the workers with the higher blood styrene levels were compared to the control group, suggesting that exposure to styrene was associated with an increased incidence of tiredness and related effects. Results from the questionnaire also indicated a marked increase in the incidence of symptoms of tiredness, but no other effects, in the styrene-exposed workers. The only significant effect noted in the behavioural studies was on reaction times. Test carried out before the work-shift indicated a statistically significantly slower reaction time in the exposed workers, a mean value of 252 $\pm$ 34 ms being obtained as compared to 230 $\pm$ 27 ms in the controls. There was no significant difference between the groups in the post-shift tests in the afternoon due to some improvement in performance of the exposed group as opposed to a slight deterioration in the controls. The mean values obtained for the exposed and control group immediately post-shift were 243 $\pm$ 29 ms and 236 $\pm$ 36 ms respectively. When the exposed group was subdivided on the basis of blood styrene levels, very little difference was noted in the morning (pre-shift) mean reaction times of the two groups. A marked difference was however noted post-shift: this was largely due to a marked improvement in performance during the shift in those workers with lower blood styrene levels, as compared to very little change over the shift in those workers with higher blood styrene levels (post-shift reaction times for workers exposed to styrene with mean blood levels above and below 5.4 mmol/l were 252 $\pm$ 27 ms and 234 $\pm$ 29 ms respectively). No significant difference was noted between the exposed and control population in most of the tests incorporated in this study. Interpretation of the pattern of result in the reaction time tests is difficult and no conclusions can be drawn for this aspect. There is some indication that occupational exposure to styrene (mean 92 ppm, 7-hour TWA) was associated with increased feelings of tiredness. However, increased tiredness may be due to increased physical activity of the individuals concerned rather than styrene exposure.

The results summarised above (Cherry *et al,* 1980) were reanalysed (Cherry *et al,* 1981). In this first study, the pre-shift reaction time results could be related to urinary mandelic acid (m.a.) concentrations after two exposure-free days in men whose m.a. was >100 mmol/mmol creatinine. A second group of 17 workers in another factory was investigated in a similar way to the previous study. The men were tested on a Monday morning, with a urine sample collected first thing that morning and further samples collected and analysed over 24 hours. This second group was only exposed to mean level of 20 ppm, range 6-31 ppm (7-hour TWA). On the Monday morning, reaction time results were noticeably slower in 2 men with high mandelic acid concentrations. The pre-shift reaction time results could be related to urinary mandelic acid in men with >50 mmol/mmol in the second factory. For the first group of workers, there was some reversibility of the slowing of reaction times after a few months of reduced exposure when those still employed had been working part time. It was shown that at least in some individual workers, metabolites of styrene are still being excreted after 2 exposure-free days. It is apparent from the toxicokinetics that investigations into any repeated-exposure effects of styrene would have to be conducted after more than a few

days free of exposure to styrene. Also, workers are typically exposed to relatively high levels of hundreds of ppm styrene during the shift but these exposures are not reported in detail.

Neurobehavioural tests were undertaken by 20 from a total of 23 male workers exposed to styrene at a Swedish factory (Edling *et al,* 1993). They had been exposed for 1-25 years and were of mean age 38. The control group of mean age 41 comprised 20 non-exposed male workers matched for age, working pattern and physical workload from nearby industries. Control and exposed groups were apparently not compared on the basis of verbal intelligence. Factors such as previous head injury and drug use were not addressed in the report. Self-reported mood, CNS and irritation and neurobehavioural symptoms were recorded using questionnaire and interaction with a computer programme. Simple reaction time, colour word vigilance (choice reaction time task) and symbol digit (perceptual motor speed) tests were administered once before work began in the morning and once after finishing work on the same day. The day of the week chosen for testing was not reported. Half of each group did their first set of tests in the morning and half in the afternoon because the authors thought that there could have been improvement effects due to practice on repeating the test. The mean 8-hour TWA styrene concentration was 8.6 ppm (range 00.4-15.4 ppm). For nine of the workers excursions greater than 50 ppm were measured. The exposed subjects reported more acute symptoms both at the beginning and end of shift but there was no change across shift. On the first recording of symptoms using the Q16 questionnaire the exposed subjects reported significantly more symptoms, particularly of tiredness and headache, than the referents. However, this could have been simply a consequence of the physical activity at work. Although the number of symptoms reported by the exposed subjects reduced when this was repeated at the end of a break from exposure for the summer holiday, improvement was statistically not significant. The change in the number of symptoms between the end and beginning of the shift was, in the exposed men, related to the 8-hr TWA for styrene. The total number of excursions above 50 ppm was related to reports of skin irritation at the end of the shift, and peak exposures were related to sensations of an unpleasant taste. In relation to the neurobehavioural tests, no difference was found on the test of simple reaction time between the exposed and referents either before or after the end of the shift. On the colour-word vigilance test (a test of complex reaction time) the exposed did worse than the referents both before and after the shift. The exposed did somewhat better, on both occasions, on the symbol digit test. No relation was found between any of the cross shift exposure measures and simple reaction time or change in simple reaction time. On the complex reaction time, scores were related to the TWA exposure, the number of excursions above 50 ppm and also to the duration of excursions above 50 ppm within the exposed group. No dose-response data were reported for the symbol digit test. Overall, no change in test scores over shift between the exposed and comparison groups was found, but within the exposed group there was a relation between complex reaction time and various measures of exposure during the shift.

No differences were observed between exposed and control groups in the results of the neurobehavioural effects at a Swedish factory where they were exposed to styrene (Flodin *et al,* 1989). There was no control group but neurobehavioural test results were compared with those of an unexposed reference group reported elsewhere. Symptoms of tiredness, dizziness, forgetfulness and headache were reported by the workers; result from other studies indicate that these general symptoms would also be reported in a control group of workers. There were no exclusions necessary on the grounds of head injury, alcohol abuse, diabetes or other factors. Following an exposure-free week, a battery of psychometric tests similar to WAIS and addressing verbal ability, cognitive functions, perceptual speed and perceptual motor speed (digit symbol), manual dexterity, learning and memory ability was administered. Exposure monitored in the breathing zone was approximately 12 ppm 8-hour TWA for nine men placed in a “high” exposure category; “low-exposed” breathed <6 ppm. Number of years potentially exposed was not given. There was a higher frequency of symptoms reported in the “high-exposure” group (mean 10.4 compared with 5.3). In the case

of 5 workers, the authors considered that the claims of abnormal fatigue and forgetfulness were exposure-related adverse effects (neurasthenic syndrome). However, these subjective symptoms were not assessed with reference to a control group and cannot be reliably attributed to styrene exposure, particularly when set in the context of findings from other studies where styrene exposures were higher. Psychometric test results were adjusted for age. When the results for the group as a whole were compared with those for the unexposed reference group, the results were within a normal range. The only statistically significant difference between the two exposure sub-groups was in the manual dexterity test for which the high exposed group had a poorer performance. After 7-9 months without exposure 17 ex-workers (from the original group of 21) were reassessed by interview; reporting of symptoms showed improvement in how the workers felt. Psychometric tests were not performed in these workers.

A personality inventory (Maudsley) and tests from the WAIS were performed by a small group of 12 GRP workers at a manufacturer in Japan (Yokoyama *et al,* 1992). The total number of potentially exposed workers and the selection procedure were not stated. The men were exposed to styrene with mean employment in lamination of 4 years. Workplace static air sampling results were mean 26 ppm $\pm$ 24 ppm, range 1-77 ppm (reference period not stated) 3 months before the study. Other solvents measured were acetone 9 ppm and methyl ethyl ketone 1 ppm. Personal airborne concentrations were estimated form biological monitoring to be in the same range as the static sampling results. Eleven male steel workers not exposed to lead or solvents were controls for the study. None of the workers in the study were taking medication or had a history of substance abuse or neurological/psychiatric disorders. The tests were picture completion (visual perception) and digit symbol (perceptual motor speed). The day of the week used for testing was not stated. Subjective symptoms of nausea, headache and local irritation were reported by some workers exposed to styrene. Analysis of covariance was used to adjust scores for differences in age, length of education and alcohol consumption between exposed and control groups. On this basis, for most tests there were no differences between the two groups. There was a statistically significantly lower score for picture completion test in the exposed group but no correlation with individual exposure levels calculated from biological monitoring results.

The reaction times of workers at four Swedish factories making plastic boats have been investigated (Gamberale *et al,* 1976). A total of 106 workers (percentage of the available exposed group not given), with an average age of 33 years were examined. The average potential duration of exposure to styrene was 2.7 years. The only exposure data were monitoring of individuals exposed over the one work-shift of the study. Contemporary mean 8-hour TWA shift values were 16-101 ppm recorded at the different plants. Some workers at one plant were exposed to 280 ppm. Each worker was subjected to a simple reaction time test in the morning before the start of the shift and immediately post-shift. The morning test was about 16 hours after the previous day's exposure to styrene. In addition, each worker was asked to assess his emotional state, using a questionnaire, at the end of the work-shift. The results obtained were compared to those found using an unexposed control group of 36 workers of comparable age (mean 32.4 years) to the exposed workers, and it was stated that both groups were comparable with respect to other variables of significance to the performance of the reaction time test. However, no further details were given, therefore the control group cannot be assumed to be adequately matched.

The results indicated that the workers exposed to styrene had longer reaction times, and showed greater impairment over the shift than the control group. Clear differences in reaction time between the two groups were noted both before the start of the shift, and its end. If this effect was related to styrene, the results suggest that some carry over in impairment had occurred during the 16 hours exposure-free period or that chronic effects were present. The difference between the two groups was however more marked at the end of the shift. In the exposed workers no significant difference was noted between the

performance times at the beginning and the end of the shift, whereas some improvement was noted over the shift in the control group. This improvement could be due to the effects of practice. No difference was noted in the emotional state of the exposed and control groups at the end of the work-shift. This study indicates that some slowing of reaction time occurred in the workers exposed to styrene over the workshift, and that some effects were observed at least 16 hours post-exposure. A dose-response relationship could not be elucidated from the available data.

Results of tests on psychological function on a very small group of 7 workers employed in the manufacture of polyester boats in Sweden have been reported (Kjellberg *et al,* 1979). A control group of seven workers from a local engineering workshop was used: these men were matched with respect to age, height and weight to the styrene-exposed workers; however no details were given as regards other factors such as pre-morbid intelligence. The personal TWA styrene concentration exposure varied in the range 3-14 ppm. The psychological tests performed on each individual consisted of a simple reaction time test, the Bourdon Wiersma test (perceptual motor speed), the R-T addition test and a subjective assessment of 'activation' level using a questionnaire. The tests for psychological function were carried out immediately prior to the work-shift (i.e. about 16 hours after the last exposure) and after the shift. In addition, studies were carried out on three subjects at 4-7 days and 35 days post-exposure, during the summer holidays.

Small differences in reaction time were noted between the exposed and the control groups. Reaction time in the control subjects was shorter in the afternoon than in the morning; this effect probably reflects improvement with practice. However, a slight deterioration rather than an improvement was noted in the exposed workers at the end of the work-shift. Taking medium reaction times over the ten-minute test period, values of 234 ms and 244 ms were noted in the styrene exposed workers at the beginning and end of the shift respectively as compared to 235 and 230 ms in the control group. In the study of three individuals examined at longer intervals post-exposure, only a very slight improvement in reaction time was noted after 4-7 days, and none after 35 days. No significant difference was noted between the two groups in any other test. Thus, in this study a slight slowing of reaction time was noted in workers exposed to average styrene levels of up to 15 ppm. However, it should be noted that there is a lack of data on the comparability of the exposed and control groups as regards potential confounding factors.

Reaction time effects were investigated in a study of Canadian workers (Cherry and Gautrin, 1990). As described in an earlier section for nerve conduction results, 70 men were exposed to styrene for between a few weeks and 20 years. There was no control group. Workers exposed to 50 ppm or above (contemporary measurements, no reference period or type of average given) and a sample of the less exposed, were invited to participate. The percentage response was not available. Exposure levels were obtained from static and personal sampling in environmental surveys. Alcohol consumption was recorded but there was no discussion of confounding factors such as pre-morbid ability, drug intake or disease. Reaction time was measured before the shift on a Monday morning and, in 28 workers, following an exposure-free (holiday) period of 3-4 weeks. Age-adjusted reaction time was not related to exposure concentration in air but was related to area under the excretion curve and to mandelic acid metabolite levels excreted on the morning of the test. In the higher exposure group (>100 ppm) employed for more than one month, reaction time was better than that expected from change due to age. The reverse was true for 5 workers exposed for less than 4 weeks. In workers with urinary metabolites detected after the weekend, reaction times were slower by 10% compared with that expected for age. An improvement in reaction time after the holiday break was seen in the workers with urinary metabolites detected after the weekend. However, overall, there was little difference in reaction times measured after the holiday break. The pattern of results seen in this study is difficult to understand.

There was no significant difference in simple reaction time results in 12 styrene-exposed men when compared with a control group of 10 men not exposed to styrene at the same Swedish factory (Edling and Ekberg, 1985). The two groups were of similar age but other potential differences such as pre-morbid ability were not discussed. The number of styrene-exposed men at the factory was not stated. Test were conducted before and after the shift on a Monday. Mean 8-hour TWA personal styrene exposure was approximately 10 and 13 ppm for the morning and afternoon shifts respectively. Mean length of exposure was 2.5 years. There was no difference in questionnaire-reported symptoms. The negative results from this study cannot be taken as conclusive because of the possible confounding differences between the groups.

Choice reaction time testing was conducted on a small number of female workers (8) before and after a 9-hour Monday workshift involving exposure to styrene (Mackay and Kelman, 1986). The total number of female workers exposed and the selection procedure were not given. Two workers not exposed to styrene were included. Information on health status, drug and alcohol use was available but other potential differences including pre-morbid intelligence was not discussed. Subjects were not exposed to other solvents. Reaction time performance improved post-shift in low/zero exposure subjects; this is likely to be a circadian rhythm effect. Reaction times were longer at the end of the shift in workers with urinary mandelic acid >500 mmol/mmol creatinine compared with controls. This could be an effect of single exposure since it was observed after the end of an 8-hour exposure. There was no effect on pre-shift results when high versus zero/low exposure workers were compared, suggesting no chronic effects of exposure.

A group of 55 male and female workers in Italy were studied to assess the effects of styrene exposure on memory, perceptual speed, attention and psychomotor function (Schoenhuber and Gentilini, 1989). All workers at factories where matabolite levels were above 700 mg/l urine in at least 5% of workers were selected for the study. Significant drug or alcohol use was excluded but other possible confounding factors (eg medical history) were not discussed. Two exposure groups were determined according to measured urinary metabolites; exposure was either above or below 700 mg/l urine. Age distribution was similar but other significant factors such as intelligence level were not addressed in the report. Workers were tested on the Monday and Friday of a working week but the time at which the test was administered was not stated. The tests were digit forward short-term memory test, symbol digit perceptual motor speed test, selective attention test (marking selected numbers from a matrix of numbers) and distributed attention tests (for visuomotor perception). For most of the tests conducted there was no difference between the two groups. A statistically significant poorer performance in the higher exposure group was observed on the Friday in the digit forward short-term memory test only. This was not found on the Monday. The digit forward test is relatively insensitive to adverse events acting on the CNS, and in view of the absence of effect in other tests there could have been significant underlying differences between the subgroups in this study.

Viaene *et al.* (1998) examined the possible influence of styrene exposure on the results of vocabulary tests between workers previously exposed to styrene and colleagues who were still exposed. One hundred and eighty-five workers, who had been employed in a styrene polyester shipbuilding factory between 1983 and 1986 and had been involved in exposure investigations by the same authors, were traced in 1992 with the aim to investigating potential chronic neurotoxic effects of styrene exposure. For economic reasons, in 1989, the older employees were made redundant leaving only a small number of subjects (31) still at work. Of the 185 workers traced, 117 were included in the study. Of these, 27 were still exposed to styrene and 90 since 1989 had ceased working with styrene. Detailed hygiene investigations had been carried out in the plant by the research group in 1983-1986. Individual records were kept for every worker on the tasks he carried out and this permitted calculation of the exact number of hours of exposure to styrene from 1983 to 1989 for the

formerly exposed workers (who were laid off at this point; a mean of 3610 hours of lamination) and to 1992 for the workers still exposed (a mean of 4700 hours of lamination). In the formerly exposed group, the median exposure to styrene during lamination tasks was 156.6 mg/m^3 (range 98.8 – 188.8 mg/m^3). This value was comparable with the exposure level of the still-exposed group (median of 147.6 mg/m^3; range 124.0 – 173.2 mg/m^3). However, at the time of the present study, the mean TWA exposure level to styrene in currently exposed workers was significantly lower (40 mg/m^3) than that measured during the period 1983-1986 (70 mg/m^3). A self-administered questionnaire was used to check for the schooling level and number of years of education. The mean length of education was 11.3 and 10.6 years in the formerly exposed and the still-exposed groups, respectively. Behavioural testing was conducted by administering the Dutch version of the vocabulary test of the Neurobehavioural Examination System (NES).

A significant relation between the vocabulary test score and duration of exposure was found. This appeared to be similar for those still exposed and those formerly exposed. No relation was found with the level of exposure. The mean vocabulary score of the still exposed group (12.5) was significantly lower than that of those who had been laid off 3 years earlier (14.3); however, in the analysis of covariance, the educational level explained a significant part of the vocabulary test results. It is noted that amongst the former workers those who agreed to take part had a higher educational level than those still employed. In view of this, it is difficult to establish whether the relation between duration of exposure and vocabulary test score observed was due to inadequate adjustment for confounding or was a real effect.

In a subsequent study by the same authors on the same formerly exposed and still-exposed groups of workers (Viaene *et al*., 2001), additional neurobehavioural tests and the persistence of complaints were investigated. A matched control group of 64 subjects with no history of occupational exposure to solvents was also included in the present study. All participants completed a self-administered questionnaire inquiring about lifestyle, medical history and education, and were interviewed about their memory of complaints while they were at work using the structured neurological anamnesis of complaints (SNAC) which investigates complaints such as headache, personality changes, concentration or memory difficulties, changed sensory functions in hand or foot, pain sensations, increased clumsiness, tremor, sleeping difficulties, diminished strength or endurance, diminished vision, hearing loss, tinnitus, altered smell, intolerance to alcohol, increased tiredness in the morning, increased tiredness in the evening, loss of equilibrium or dizziness. The subjects also completed a 60-item questionnaire (the neurotoxicity symptom checklist) for screening complaints at the time of the study (1992-1994), and were administered a neurobehavioural test battery including tests for hand-eye coordination, simple reaction time, symbol-digit substitution to assess visuomotor performance, associated learning, associated recall, digit span forward and colour-word vigilance to assess concentration and memory. Blood samples were also collected to characterise the microsomal epoxide hydrolase (mEH) activity in mononuclear cells.

Despite efforts to match the groups according to educational level, substantial differences were found amongst the currently exposed workers (the lowest level), the formerly exposed group (an intermediate level) and the control subjects (the highest level). It is also interesting to note that the education level of the workers exposed to styrene who did not participate was even lower than that of the participating currently exposed group. For the period before 1989 (i.e. before exposure conditions improved in the plant), currently and formerly exposed workers remembered having more complaints at work (irritability, fatigue in the evening, headache, common colds, intolerance for alcohol, diminished endurance, memory and sense of smell) than the control group did, which related well with the mean exposure to airborne styrene concentration. Most complaints disappeared after the end of exposure, however, 10 of the formerly exposed subjects, despite not having been exposed for 3 years, still complained at the time of the SNAC questionnaire of a persistently diminished sense of smell. Full details of the results of the 60-item questionnaire are not given, but it appears that

the formerly exposed workers had worse scores than the control subjects for the chest, equilibrium and somatic categories. Currently and formerly exposed workers performed worse than control workers in the symbol-digit substitution and digit span forward tests, however, the education level explained a significant part of the symbol-digit substitution results. Hand-eye coordination was significantly worse only in formerly exposed workers compared with control workers. All the other neurobehavioural test results did not differ significantly between the groups. In the combined group of currently and formerly exposed workers, the symbol-digit substitution and colour-word vigilance results related well to duration of exposure. A five-fold variation in mEH activity was found among the studied population. In a model including duration of exposure, unexpectedly, the individuals with higher mEH activity (faster detoxification) did worse on the symbol-digit substitution test, on the colour-word vigilance test and on the digit span forward test. The interpretation of the higher incidence of remembering symptom complaints at work by the exposed workers compared to the control group is rather difficult, as without an analysis of changes over the course of a shift, a clear relationship with styrene exposure cannot be established. Furthermore, the collection of symptom complaints from a group of workers who had been laid off for 3 years, and for whom the method of parting may not have been ideal, raises serious questions of interpretation. Thus, the persistent diminished sense of smell reported by 10 formerly exposed workers is considered to be of doubtful reliability. The data from the behavioural testing are also difficult to interpret because of inadequate adjustment/matching for the educational level. It is interesting to note that, although vocabulary test data were available for the exposed groups from the previous investigation (Viaene *et al*., 1998), no adjustment for vocabulary score was attempted by the authors.

In a cross-sectional study of 213 boat builders exposed to styrene and 114 non-exposed employees from the same company, alterations of the Profile of Mood States (POMS) were investigated (Challenor and Wright, 2000). From passive samples of 23 subjects obtained 2 weeks after completing the study, the highest air concentration of styrene measured in the plant was 66 ppm. A PMOS questionnaire was administered, asking the subject to rate feelings during the past week, “including today”. A supplementary questionnaire on job satisfaction, life events, stress, smoking habits and alcohol consumption was also completed.

The styrene exposed workers had scores on the aggression scale of the POMS that suggested they were more aggressive than the non-exposed. This difference was marginally significant. Further analysis suggested that this aggression was most evident in those who had started working with styrene more recently. It is noted that the styrene exposed group smoke more than the control group. Overall, this study provides a description of a phenomenon noted in a single factory, and it is not clear that it can be generalised to other situations.

As indicated in the introduction to this section, interpretation of these studies in terms of any potential long-term effects of styrene exposure is complicated by the fact that in the majority of the studies, testing was conducted on workers who had been recently exposed to styrene. Thus the possibility that any neurobehavioural changes reported are related to an acute effect of exposure cannot be excluded. However, it may be that a clearer pattern of results would emerge from studies in which any possible short-term effects can be ruled out. The data from Cherry *et al,* (1981) indicate that urinary metabolites of styrene may still be present in workers following 2 exposure-free days. Thus, to exclude results that might reflect a potential short-term effect of styrene exposure, only studies in which testing was performed following an exposure break of more than 2 days should be considered. Of the 25 different studies described in this section, only four report that testing was performed in subjects following an exposure-free period of more than 2 days: Flodin *et al,* (1989) performed a psychometric test battery in subjects after a week with no exposure; Cherry and Gautrin (1990) tested workers at 3-4 weeks after the last exposure; and Viaene *et al.* (1998

and 2001) tested workers who had been laid off 3 years earlier. Flodin *et al,* (1989) found a statistically significant deficit in a single test (for manual dexterity) in a 'high' exposure group (12 ppm 8-hour TWA) compared with a 'low' exposure group (<6 ppm 8-hour TWA). In the study by Cherry and Gautrin (1990), reaction time was assessed in 28 workers following a 3-4 week break from exposure. There was no significant change in age-adjusted reaction time for the group as a whole when tested after the break compared with before the break, although there was some evidence for a slowing of reaction time related to acute styrene exposure. In the study by Viaene *et al*. (1998), the mean vocabulary score of the still exposed group was significantly lower than that of those who had been laid off 3 years earlier, and in Viaene *et al*. (2001) hand-eye coordination was significantly worse in the formerly exposed workers (those who had been laid off 3 years earlier) compared with control workers. However, these data are difficult to interpret because of the inadequate adjustment/matching for educational level.

Overall, there is insufficient information on which to base any conclusions about the potential long-term effects of styrene exposure on neurobehavioural parameters. There are only four studies in which the potential for acute effects can be entirely excluded. One of these studies suggests a possible deficit in a single neurobehavioural parameter (manual dexterity); another provides no evidence for a functional deficit in reaction time; and the two most recent studies (Viaene et al., 1998 and 2001) failed to demonstrate that the effects seen were due to styrene exposure because of inadequate adjustment/matching for educational level.

Benignus et al. (2005) conducted a meta-analysis of several studies that have reported on the relationship between styrene exposure and effects on reaction time (simple and choice) and colour vision. Four studies of choice reaction time (CRT), 3 studies of simple reaction time (SRT) and 5 studies of colour vision discrimination were selected. No clear inclusion/exclusion criteria for the selected studies were provided. Airborne styrene concentrations were estimated from urinary styrene or metabolite data by fitting regression equations. No standard extrapolations were used. Cumulative exposures were then calculated as the product of concentration and time by assuming that the current exposure concentration was a representative measure of the workers' exposure history. In the interest of homogeneity, study group means for both concentration and duration of exposure rather than individual data were computed. Outcome data were transformed to a common metric of effect magnitude (percentage of baseline) and linear equations were fitted to the pooled data to produce dose-response relationships. In the majority of the reports, it is unclear whether or not the different tests were administered in a blind manner. Statistically significant relationships were found between cumulative styrene exposure and increased choice reaction time as well as increased colour confusion index (CCI). Eight years of exposure to 20 ppm styrene was estimated to produce a 6.5% increase in CRT and to increase the CCI as much as 1.7 additional years of age in men. No significant effects were observed on SRT.

It is considered that a number of methodological weaknesses in the analysis question the validity and reliability of these findings. First of all, it is noted that the meta-analysis presented failed to reflect the available literature, as several additional studies could have been included in the model calculations, but were not. Secondly, given that study group means for both concentration and duration of exposure rather than individual data were used and that there was no information on past exposure, the dose-response and time-response evaluations presented are likely to be unreliable. Thirdly, by using percentages of baseline response, the variability of the control groups was unaccounted for. Fourthly, by imposing a linear mathematical model to the dose-response relationships, the study failed to reflect the available data and the threshold nature of these effects. Also, it is difficult to understand the plausibility as to why CRT is affected but not SRT; such a specific effect is of doubtful toxicological significance.

Summary of results from neurobehavioural tests

Volunteer studies indicate that a single exposure to styrene levels of 200 ppm for 90 minutes or 350 ppm for 30 minutes can produce some slight reduction in performance in reaction time tests (a slower response time) and other similar parameters. These effects are related to the general CNS depressant potential of styrene (see single exposure section).

Numerous workplace studies using neurobehavioural testing are available. It is noted that a range of significant confounding factors has not always been addressed. The results obtained have been variable, with some studies reporting effects and others no effects for workers within similar exposure ranges. Performance was adversely affected in several studies in only a small proportion (1-3 of the 6-20) of the tests administered. Also, different types of neurobehavioural test (representing various functional domains of the CNS) were apparently sensitive to styrene exposure in different studies. This lack of a clearly consistent effect on particular functional domains indicates that there is only weak evidence for a causal relationship. In some studies, there is a possibility that effects similar to those detected in volunteer studies i.e. slightly slower response times were observed and these might be related to brief peaks of styrene exposure of hundreds of ppm that had occurred during the previous shift. Overall, there is insufficient information on which to base any conclusions about the potential long-term effects of styrene exposure on neurobehavioural parameters. There are only four studies in which the potential for acute effects can be entirely excluded. One of these studies suggests a possible deficit in a single neurobehavioural parameter (manual dexterity); another provides no evidence for a functional deficit in reaction time; and the two most recent studies (Viaene et al., 1998 and 2001) failed to demonstrate that the effects seen were due to styrene exposure because of inadequate adjustment/matching for educational level. In view of this, it is not possible to discern a clear dose-response relationship for any of the effects observed in neurobehavioural tests in workers.

Furthermore, at present there is not a clearly established, widely accepted interpretational framework into which these results can be fitted. Where apparent deficits in neurobehavioural test performance have been measured, the underlying toxicological processes involved, the consequences for the health and safety of the individual, and the effect of styrene in comparison with that of other experiences and phenomena regularly encountered in everyday life, have not been established.

Taking all of these points into consideration, the rapporteur proposes that the crucial issue in relation to the impact of styrene on the nervous system is the need to avoid acute CNS depressant effects and associated symptomatology.

Summary of all neurological studies

Because styrene is highly lipid-soluble and, like many other organic solvents, at certain concentrations, produces acute CNS effects, concern about the long-term toxicity of styrene has been focused on its potential for damaging the nervous system. To that end, for the last 40-45 years, several studies investigating the potential neurotoxicity of styrene in exposed workers have been undertaken in factories in many parts of the world. The majority of these studies have suggested that styrene has substantive effects on the nervous system in humans such that the generally uncritical recitation of the results from these investigations has created over the years the label that styrene is a potent neurotoxicant. However, despite this extensive investigation of styrene potential neurotoxicity, a critical review of the available data has shown that there is no clear relationship between repeated exposure to styrene and persisting damage to the nervous system.

In an European cohort study, an association was found within the cohort between increasing levels of styrene exposure and mortality from CNS diseases. However, overall mortality from CNS disease was lower than the national rate and there were only relatively small numbers

of deaths from any given cause within this category. Also the follow-up was very short with only 7% of deaths within the cohort and there was a lack of information on important workplace or lifestyle confounders. Furthermore, no increase in mortality from CNS diseases was found in an US cohort. Without further confirmation, the results of the European cohort study do not provide convincing evidence of an effect of styrene exposure.

Several studies have been conducted on EEG patterns in styrene-exposed workers. Overall, the collective findings do not provide robust evidence for the absence or presence of styrene-induced EEG changes in exposed workers. Furthermore, although the approach is valuable in that it provides measures of nervous system function that are independent of the level of collaboration of the subject, no clear criteria for interpretation of the health significance of any EEG changes that might have occurred are available. If styrene was the causal agent of any EEG changes in workers covered by these studies, the most likely interpretation is that the effects were due to the general CNS depressant action of styrene.

The available nerve conduction studies have produced inconsistent results for different groups of workers exposed to similar levels of styrene. Some studies have indicated a correlation between styrene exposed and small (>10%) decreases in nerve conduction velocity, compared with unexposed controls. Others have shown similar differences in nerve conduction velocity between styrene-exposed and unexposed workers. Overall, it is not clear if styrene exposure can produce a decrease in nerve conduction velocity. Furthermore, if it can, the underlying basis for the effect could not be deduced from the available information. Also the clinical significance of this effect is questionable as all subjects appeared to be healthy workers.

A few studies are available which were designed to investigate any effect of styrene on hearing function. When confounding factors such as age and noise exposure were taken into account, no relationship between styrene exposure and hearing loss was found. There is limited evidence that styrene exposure may have caused minor effects on vestibular reflexes in some workers. However, the quality of the exposure data is such that it is not possible to relate these effects to reliable levels of styrene exposure. Therefore, although these human data cannot be used for risk characterisation purposes, nevertheless they indicate that the observations of ototoxicity in animals may be relevant to humans.

In one study designed to investigate olfactory function, other than an adaptive increase in the threshold for odour detection of styrene itself, no olfactory deficit was evident in workers exposed to up to 77 ppm (8-hour TWA).

Studies on colour vision provide some evidence to support the view that styrene does cause changes in colour discrimination relative to age-matched controls. However, it should be noted that, as a single colour vision test rather than a testing battery approach was used, these findings are not sufficiently robust to reliably characterise the scale and the nature of the effect. Generally, the effect was on the tritan (blue-yellow) type, although some workers also had evidence of red-green colour vision deficiency. However, there is a lack of information on whether the effect is related to short-term or repeated long-term exposures to styrene. There is also a lack of information on the magnitude of the effect in workers exposed to styrene concentrations > 100 ppm (8h TWA), although performance in colour discrimination tests at these high exposures is likely to be subject to confounding because of transient CNS depression or eye irritation. Studies suggest that changes in colour discrimination would not be expected with 8 h TWA exposures below 20 ppm. However, although there are findings suggesting minor changes in colour discrimination at relatively high levels of exposure to styrene (≥ 50 ppm, 8h TWA), it should be noted that the available studies are not sufficiently robust to reliably characterise the scale and the nature of the effect. Also, given the very mild nature (the affected individuals were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, it is deemed that the slight changes in colour discrimination detected should not be considered as an adverse health outcome of styrene

exposure. It can be concluded that, since the effects observed at 50 ppm (216 mg/m^3; 8h TWA) are not yet adverse, this exposure value can be considered a NOAEC.

The results of two biochemical studies by one research group, taken together, are rather inconsistent and the inconsistency increases when a further study by different authors is considered. Overall, there are indications of lower levels of serum dopamine β-hydroxylase activity and higher levels of serum prolactin (PRL) with styrene exposure (mean levels between 25 and 58 ppm). There are also indications that increased PRL secretion is an acute rather than a chronic effect of styrene exposure. However, the evidence is not robust, an underlying mechanism not clearly established and the clinical consequences not characterised. Furthermore, the importance of these results in the understanding of neurotoxicity has yet to be established; also, in comparing exposed to a non-exposed comparison group the problems of unmeasured confounding is substantial because the relevant confounders are unknown. Overall, it is suggested that these findings should not be used in characterising the toxicological hazard assessment of styrene.

Volunteer studies indicate that a single exposure to styrene levels of 200 ppm for 90 minutes or 350 ppm for 30 minutes can produce some slight reduction in performance in reaction time tests (a slower response time) and other similar parameters. These effects are related to the general CNS depressant potential of styrene. Numerous workplace studies using neurobehavioural testing are available. It is noted that the range of significant confounding factors has not always been addressed. The results obtained have been variable, with some studies reporting effects and others no effects for workers within similar exposure ranges. Performance was adversely affected in several studies in only a small proportion (1-3 of the 6-20) of the tests administered. Also, different types of neurobehavioural test (representing various functional domains of the CNS) were apparently sensitive to styrene exposure in different studies. This lack of a clearly consistent effect on particular functional domains indicates that there is only weak evidence for a causal relationship. In some studies, there is a possibility that effect similar to those detected in volunteer studies i.e. slightly slower response times were observed and these might be related to brief peaks of styrene exposure of hundreds of ppm that had occurred during the previous shift. Overall, there is insufficient information on which to base any conclusions about the potential long-term effects of styrene exposure on neurobehavioural parameters. There are only four studies in which the potential for acute effects can be entirely excluded. One of these studies suggests a possible deficit in a single neurobehavioural parameter (manual dexterity); another provides no evidence for a functional deficit in reaction time; and the two most recent studies (Viaene et al., 1998 and 2001) failed to demonstrate that the effects seen were due to styrene exposure because of inadequate adjustment/matching for educational level. In view of this, it is not possible to discern a clear dose-response relationship for any of the effects observed in neurobehavioural tests in workers. Furthermore, at present there is not a clearly established, widely accepted interpretational framework into which these results can be fitted. Where apparent deficits in neurobehavioural test performance have been measured, the underlying toxicological processes involved, the consequences for the health and safety of the individual, and the effect of styrene in comparison with that of other experiences and phenomena regularly encountered in everyday life, have not been established.

Taking all of these points into consideration, the rapporteur propose that the crucial issue in relation to the impact of styrene on the nervous system is the need to avoid acute CNS depressant effects and associated symptomatology.

4.1.2.6.3 Summary of Effects of Repeated Exposure

There is a large amount of information from studies in humans. The authors of the few studies that have reported on patterns of mortality from non-malignant disease in occupational groups exposed to styrene have signalled findings that have been proposed to

be worthy of further exploration. However, a critical appraisal of the studies, taken together, suggests that they present no convincing evidence that styrene exposure has enhanced the incidence of mortality from any particular disease.

In the available worker health survey studies, consistent evidence of an increase in the self-reporting of symptoms of eye and nasal irritation and CNS disturbance (drowsiness, headache, lightheadedness) comes through. Unfortunately, the quality of the exposure data means that it is not possible to relate these effects to reliable levels of styrene exposure, particularly as it is possible that these effects are related more to short-term peaks in exposure, rather than to workshift averages. No reliable evidence for any other effects of styrene is indicated by these studies. From the studies available there is no convincing evidence of a clear, interpretable and toxicologically significant effect of styrene having occurred in exposed workers, in relation to haematological, immunological, hormonal or renal endpoints.

Because styrene is highly lipid-soluble and, like many other organic solvents, at certain concentrations, produces acute CNS effects, concern about the long-term toxicity of styrene has been focused on its potential for damaging the nervous system. To that end, for the last 40-45 years, several studies investigating the potential neurotoxicity of styrene in exposed workers have been undertaken in factories in many parts of the world. The majority of these studies have suggested that styrene has substantive effects on the nervous system in humans such that the generally uncritical recitation of the results from these investigations has created over the years the label that styrene is a potent neurotoxicant. However, despite this extensive investigation of styrene potential neurotoxicity, a critical review of the available data has shown that there is no clear relationship between repeated exposure to styrene and persisting damage to the nervous system.

In an European cohort study, an association was found within the cohort between increasing levels of styrene exposure and mortality from CNS diseases. However, overall mortality from CNS disease was lower than the national rate and there were only relatively small numbers of deaths from any given cause within this category. Also the follow-up was very short with only 7% of deaths within the cohort and there was a lack of information on important workplace or lifestyle confounders. Furthermore, no increase in mortality from CNS diseases was found in an US cohort. Without further confirmation, the results of the European cohort study do not provide convincing evidence of an effect of styrene exposure.

Several studies have been conducted on EEG patterns in styrene-exposed workers. Overall, the collective findings do not provide robust evidence for the absence or presence of styrene-induced EEG changes in exposed workers. Furthermore, although the approach is valuable in that it provides measures of nervous system function that are independent of the level of collaboration of the subject, no clear criteria for interpretation of the health significance of any EEG changes that might have occurred are available. If styrene was the causal agent of EEG changes in workers covered by these studies, the most likely interpretation is that the effects were due to the general CNS depressant action of styrene.

The available nerve conduction studies have produced inconsistent results for different groups of workers exposed to similar levels of styrene. Some studies have indicated a correlation between styrene exposure and small (<10%) decreases in nerve conduction velocity, compared with unexposed controls. Others have shown similar differences in nerve conduction velocity between styrene-exposed and unexposed workers. Overall, it is not clear if styrene exposure can produce a decrease in nerve conduction velocity; furthermore, if it can, the underlying basis for the effect could not be deduced from the available information. Also the clinical significance of this effect is questionable as all subjects appeared to be healthy workers.

A few studies are available which were designed to investigate any effect of styrene on hearing function. When confounding factors such as age and noise exposure were taken into account, no relationship between styrene exposure and hearing loss was found. There is limited evidence that styrene exposure may have caused minor effects on vestibular reflexes in some workers. However, the quality of the exposure data is such that it is not possible to relate these effects to reliable levels of styrene exposure. Therefore, although these human data cannot be used for risk characterisation purposes, nevertheless they indicate that the observations of ototoxicity in animals may be relevant to humans.

In one study to investigate olfactory function, other than an adaptive increase in the threshold for odour detection of styrene itself, no olfactory deficit was evident in workers exposed to up to 77 ppm (8-hour TWA).

Studies on colour vision provide some evidence to support the view that styrene does cause changesin colour discrimination relative to age-matched controls. However, it should be noted that, as a single colour vision test rather than a testing battery approach was used, these findings are not sufficiently robust to reliably characterise the scale and the nature of the effect. Generally, the effect is on the tritan (blue-yellow) type, although some workers also had evidence of red-green colour vision deficiency. However, there is a lack of information on whether the effect is related to short-term or repeated long-term exposures to styrene. There is also a lack of information on the magnitude of the effect in workers exposed to styrene concentrations > 100 ppm (8h TWA), although performance in colour discrimination tests at these high exposures is likely to be subject to confounding because of transient CNS depression or eye irritation. Studies suggest that changes in colour discrimination would not be expected with 8 h TWA exposures below 20 ppm. However, although there are findings suggesting minor changes in colour discrimination at relatively high levels of exposure to styrene ($\geq$ 50 ppm, 8h TWA), it should be noted that the available studies are not sufficiently robust to reliably characterise the scale and the nature of the effect. Also, given the very mild nature (the affected individuals were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, it is deemed that the slight changes in colour discrimination detected should not be considered as an adverse health outcome of styrene exposure. It can be concluded that since the effects observed at 50 ppm (216.5 mg/m^3; 8h TWA) are not yet adverse, this exposure value can be considered a NOAEC against which risk characterisation should be performed.

The results of two biochemical studies by one research group, taken together, are rather inconsistent and the inconsistency increases when a further study by different authors is considered. Overall, there are indications of lower levels of serum dopamine β-hydroxylase activity and higher levels of serum prolactin (PRL) with styrene exposure (mean levels between 25 and 58 ppm). There are also indications that increased PRL secretion is an acute rather than a chronic effect of styrene exposure. However, the evidence is not robust, an underlying mechanism not clearly established and the clinical consequences not characterised. Furthermore, the importance of these results in the understanding of neurotoxicity has yet to be established; also, in comparing exposed to a non-exposed comparison group the problems of unmeasured confounding is substantial because the relevant confounders are unknown. Overall, it is suggested that these findings should not be used in characterising the toxicological hazard assessment of styrene. Volunteer studies indicate that a single exposure to styrene levels of 200 ppm for 90 minutes or 350 ppm for 30 minutes can produce some slight reduction in performance in reaction time tests (a slower response time) and other similar parameters. These effects are related to the general CNS depressant potential of styrene.

Numerous workplace studies using neurobehavioural testing are available. It is noted that the range of significant confounding factors has not always been addressed. The results

obtained have been variable, with some studies reporting effects and others no effects for workers within similar exposure ranges. Performance was adversely affected in several studies in only a small proportion (1-3 of the 6-20) of the tests administered. Also, different types of neurobehavioural test (representing various functional domains of the CNS) were apparently sensitive to styrene exposure in different studies. This lack of a clearly consistent effect on particular functional domains indicates that there is only weak evidence for a causal relationship. In some studies, there is a possibility that effects similar to those detected in volunteer studies ie slightly slower response times were observed and these might be related to brief peaks of styrene exposure of hundreds of ppm that had occurred during the previous shift. Overall, there is insufficient information on which to base any conclusions about the potential long-term effects of styrene exposure on neurobehavioural parameters. There are only four studies in which the potential for acute effects can be entirely excluded. One of these studies suggests a possible deficit in a single neurobehavioural parameter (manual dexterity); another provides no evidence for a functional deficit in reaction time; and the two most recent studies (Viaene et al., 1998 and 2001) failed to demonstrate that the effects seen were due to styrene exposure because of inadequate adjustment/matching for educational level. In view of this, it is not possible to discern a clear dose-response relationship for any of the effects observed in neurobehavioural tests in workers. Furthermore, at present there is not a clearly established, widely accepted interpretational framework into which these results can be fitted. Where apparent deficits in neurobehavioural test performance have been measured, the underlying toxicological process involved, the consequences for the health and safety of the individual, and the effect of styrene in comparison with that of other experiences and phenomena regularly encountered in everyday life, have not been established.

Taking all of these points into consideration, the rapporteur proposes that the crucial issue in relation to the impact of styrene on the nervous system is the need to avoid acute CNS depressant effects and associated symptomatology.

In relation to findings in experimental animals, a variety of repeated inhalation exposure studies in different animal species are available. However, among these species, the rat and mouse have been the most extensively investigated. Four well characterised target sites of toxicity have been identified: the nasal epithelium (in rats and mice), the lung (in the mouse), the liver (in the mouse) and the ear (in the rat).

In relation to nasal epithelium damage, a NOAEC has not been identified, with chronic inflammatory changes (mild in degree and confined to the olfactory epithelium with no effects on Bowman's glands or olfactory nerve fibres), having been seen in the rat with long term daily exposure to 50 ppm styrene, the lowest concentration explored in a 2-year study. Nasal toxicity has also been reported in mice. In a similar 2-year study, respiratory metaplasia of the olfactory epithelium, hyperplasia and hypertrophy of the Bowman's gland and atrophy of the olfactory nerve fibres were observed starting from 20 ppm, the lowest concentration tested. It is clear that the nasal lesions induced by styrene exposure are a lot more severe in the mouse compared to the rat. Over the years a number of investigative studies have been undertaken to characterise and explain these species differences and to investiagte the relevance of these findings to humans. The results of these investigations have shown that the differences in nasal toxicity between rat and mouse can be explained by the greater ability of the rat nasal epithelium to detoxify reactive metabolites of styrene formed *via* CYP2F2 metabolism. These reactive/toxic intermediates include styrene oxide and most probably the downstream metabolites of 4-VP. Detoxification of toxic species by epoxide hydrolase is 10-fold higher in the rat olfactory tissue as compared to the mouse while glutathione S-transferase activity is approximately 3-fold higher in the rat nasal tissue as compared to the mouse. It is important to consider that cytochrome P450-catalysed metabolic activation of styrene to styrene oxide in human nasal epithelial tissue appears to be negligible (see toxicokinetics section). Also, since uptake of styrene by nasal tissue is

enhanced by the ability of the tissue to metabolize styrene, the lack of the primary step of styrene metabolism in human nasal tissue serves as a further reason for dismissing the relevance to humans of these rodent nasal lesions. Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which result in significant different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is likely that high levels will not be deposited in the olfactory area. Furthermore, human investigations have shown that exposure up to 77 ppm (8h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function, and in several human health surveys of workers repeatedly exposed to styrene up to approximately 700 ppm, no nasal lesions have been described (see RDT, human studies section). Hence, it can be concluded that rodent nasal epithelium damage induced by styrene is not of relevance in relation to the potential repeated dose effects of styrene in humans at relevant levels of exposure.

In relation to the lung, no effects were seen in rats exposed up to 1000 ppm, but in mice a NOAEC was not identified, with damage to the lung epithelium (hyperplasia in the terminal bronchioles, focal bronchiolar-alveolar hyperplasia, fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct) having been seen from 20 ppm, the lowest exposure concentration tested in a 2-year study. Work with the cytochrome P450 inhibitor 5-P-1-P has shown that metabolic activation of styrene to styrene oxide and other reactive metabolites (e.g the downstream metabolites of 4-vinylphenol) and the subsequent detoxification of styrene oxide are crucial elements of this toxic response. This is supported by the observation that it is the metabolically active Clara cells that are the initial focus of damage. These non-ciliated bronchiolar epithelial cells are mainly involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium (Komaromy and Tigyi, 1988). Early biochemical changes, sustained cell damage and regenerative cell proliferation were observed in lung Clara cells of mice exposed to 40 and 160 ppm styrene for up to 4 weeks. In this context it is important to consider that cytochrome P450-catalysed metabolic activation of styrene in human lung tissue appears to be minimal (see toxicokinetic section) even though the two main P450 isoforms involved in styrene metabolism, CYP2E1 and CYP2F1, have been detected; that the number of Clara cells in human lung is very low; and that their most important functions in human lung are shared by other cell types (e.g. Type II cells). Hence, it can be concluded that these lung tissue findings in mice reflect a toxic response that will not occur to any significant extent in humans at relevant levels of exposure.

In relation to the liver, exposures in the range 150-350 ppm have produced fatal hepatotoxicity in mice. Again, it appears that the metabolic activation of styrene to styrene oxide is a crucial stage in the hepatotoxicity process. In this context, it is evident that the toxicokinetic information available suggests that the mouse would be more susceptible than humans to styrene induced hepatotoxicity. This is borne out by the observation of mouse fatalities at exposure levels to which humans have been regularly exposed in the past, with no reports of hepatotoxicity or death.

In relation to effects on the ear, clear evidence of ototoxicity (both functional and histological has been seen in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks' duration. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tend to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm. Comparative studies using

rats and guinea pigs exposed to 1000 ppm for 5 days indicate an obvious species-difference, as similar findings were not observed in guinea pigs. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. The underlying toxicological mechanism has not been clearly elucidated. This effect should be therefore regarded as of potential relevance to human health. The histological damage consists in the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irriversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organisation of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. On this basis, it is considered that the NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively are a more accurate starting point for risk characterisation than the NOAEC of 200 ppm for 13 weeks.

In one single non-standard investigation, rather minor effects on the number of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks have been reported. However, it is considered that, in the absence of any associated conventional histopathology and given the lack of infromation on what are the ‘normal’ levels of dopamine and glutathione in the retinal cells of rats, the rather minor morphometric and biochemical changes observed are unlikely to represent a toxic response.

Overall, the available inhalation repeated dose toxicity studies have identified ototoxicity as the most sensitive and relevant effect of styrene repeated inhalation exposure with NOAEC values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) for 4 weeks in sedentary/ordinary and active rats respectively.

Most of the available repeated oral exposure studies have been performed in rats and mice. Information from a carcinogenicity bioassay in the rat has shown no evidence of treatment related toxicity in animals administered 1000 mg/kg/day for two years; a marked increase in mortality was evident at 2000 mg/kg/day. In another 2-year study, styrene did not produce any clear evidence of toxicity when administered in drinking water at a dose of 21 mg/kg/day, the highest dose level tested. However, it is noted that potential effects on the ear were not investigated in these studies. Ototoxicity, a clearly established effect of styrene in the rat, was seen in one study at 800 mg/kg/day (and perhaps, although less convincingly, at 400 mg/kg/day) for 2 weeks.

In mice, the most reliable information comes from a cancer bioassay, in which increased mortality and hepatic necrosis were observed at the highest dose of 300 mg/kg/day; a NOAEL of 150 mg/kg/day was identified from this study. The one significant observation from the remaining studies is that of toxicity towards the lung epithelium, adding further support to the concept that the lung toxicity of styrene in mice, following oral and inhalation exposure, results from local metabolism of styrene to styrene oxide and to other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol).

Overall, in relation to repeated oral exposure, the NOAEL of 150 mg/kg/day identified from a 2-year cancer bioassay in the mouse should also be considered. But in extrapolation to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat.

No repeated dermal studies are available, although low systemic toxicity would be predicted in most conventional experimental species with the possible exception of some strains of the mouse.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in vitro

Bacterial studies

Many bacterial studies in Salmonella typhimurium have been conducted with styrene and its metabolites, both in the presence and absence of exogenous metabolic activation. Generally, bacterial mutagenicity tests have been conducted using liquid styrene and principally in S. typhimurium strains TA100 and TA1535, although some studies have also been conducted using strains TA98, TA1537 and TA1538. Conflicting results have been obtained in these studies. Negative results have been obtained with styrene both in the absence (Milvi *et al,* 1976 and De Meester *et al,* 1977b) and presence (Vainio *et al,* 1976; Stoltz *et al,* 1977; De Meester *et al,* 1977a; Loprieno *et al,* 1978a; Watabe *et al,* 1978a, Watabe *et al,* 1978b; Busk *et al,* 1979; Bauer *et al,* 1980a and 1980b; Poncelet *et al,* 1980, De Flora, 1981; De Flora *et al,* 1984; Dunkel *et al,* 1985; Brams *et al,* 1987, and Brunnemann *et al*, 1992) of exogenous metabolic activation (induced or uninduced rat, mouse or hamster liver S9) at up to cytotoxic concentrations. However, positive results have also been reported, in the presence of induced rat liver S9 (Vainio *et al,* 1976; De Meester *et al,* 1977; Bauer *et al,* 1980a and 1980b; Poncelet *et al,* 1980; De Meester *et al,* 1981; and Brams *et al,* 1987). Considering that there are inconsistencies in the results obtained from study to study, it is difficult to determine whether the conflicting results observed were due to impurities, the metabolic system employed or other methodological differences.

Dolara *et al,* (1984), reported that urine obtained from workers exposed to styrene was not mutagenic in a bacterial Ames test *in vitro* using S. typhimurium strains TA100, TA1535 and TA1538.

Styrene was found to be negative in forward (MTR) and back-mutation systems (gal- arg- and nad-) in Escherichia coli in the presence of phenobarbital or clophen-induced mouse liver S9 (Ellemberger *et al,* 1975 and Grein *et al,* 1977). Negative results were also reported in an E.coli WP2uvrA reverse mutation assay (Norrpa *et al,* 1985), even in the presence of an activation system of human erythrocytes.

De Flora (1984), did not obtain any evidence of styrene-induced DNA damaging activity in several DNA-repair-proficient and –deficient E.coli strains (WP2, WP67, CM817) in the absence or presence of an exogenous metabolic activation system.

Styrene also gave negative results with the SOS chromotest in the presence of S9 over a concentration range of 10 ng to 1 mg/ml (Brams *et al,* 1987).

Overall, although a number of studies involving bacterial test systems have yielded negative results, the positive findings in Ames tests produced by several groups, using exogenous metabolic activation systems, suggest that styrene can become metabolically activated to a bacterial mutagen *in vitro*.

Yeast studies

Styrene produced negative results in yeast forward mutation and mitotic gene conversion assays in Schizosaccharomyces pombe (P1 strain) and Saccharomyces cerevisiae (D4 strain) in the presence and absence of exogenous metabolic activation at concentrations up to 100 mM (Loprieno *et al*, 1976). However, some mutagenic activity was observed in logarithmically growing S. cerevisiae D7 which have metabolic activity (Del Carratore *et al*, 1983).

Styrene has been investigated in host-mediated assays, with S. cerevisiae or S. pombe as marker micro-organisms for gene conversions or point mutations respectively (Loprieno *et al,* 1976). The micro-organisms were given by the intraperitoneal route to mice. Styrene was given orally at 1000 mg/kg and the micro-organisms were harvested 1, 3 and 6 hours post-dose. An increase in gene conversions, with no significant increase in forward mutations, was noted but the authors considered the outcome of both elements of this assay to be negative, when a larger set of control values was taken into account (Loprieno and Abbondandolo, 1980).

Mammalian cell studies

Negative results were produced when styrene was tested in a forward mutation hypoxanthine-guanine phosphoribosyl transferase (*HPRT)* assay (with and without mouse S9) in Chinese hamster V79 cells (Loprieno *et al*, 1976, Loprieno *et al*, 1978). Styrene was tested at up to 51 mM. Some evidence of mutagenic activity was suggested in V79 cells when a styrene treated rat liver perfusate was tested (Beije and Jensen, 1982). However this study was unconventional and not a thorough one, and both control and treated results were variable; no firm conclusions can be drawn.

In a poorly reported *in vitro* chromosome aberration study, cultured Chinese hamster lung cells were treated with styrene at 2.4 mM for 3 hours in the presence of a liver metabolising system (Matsuoki *et al,* 1979). The cells were harvested 24 hours later and the incidence of cells with aberrations, including gaps, was high (19%). In the absence of metabolic activation, the incidence was 2%. No figures were given on the relative incidence of each type of aberration; control data were not provided and no cytotoxicity information was given. Therefore it is not possible to draw any firm conclusions from this study.

In another *in vitro* chromosome aberration study, styrene was incubated with cultured human lymphocytes at 0.005 – 0.5 mM for 24 hours before harvest (Pohlova *et al,* 1985). A statistically significant increase to 5% incidence of cells with chromosome aberrations, excluding gaps, was observed at the highest concentration only. The vehicle control frequency was 1.5%. No exogenous metabolic activation was used. There was no information on cytotoxicity in the report, making the interpretation of this study difficult; again, no firm conclusions can be drawn.

A further assay of this type used a range of styrene concentrations of 0.5 – 6.0 mM, incubated with human lymphocytes in whole blood culture for 24 hours, with no added metabolic activation system (Jantunen *et al,* 1986). A statistically significant, concentration-related increase to 19.5 chromosome aberrations per 100 cells, excluding gaps, was observed. A smaller statistically significant increase occurred in isolated lymphocyte cultures. In this type of assay, the results should be reported in terms of the frequency of aberrant cells, not the frequency of aberrations in total. Nevertheless, the result is strongly suggestive of mutagenic activity.

Lastly, in relation to chromosome aberration assays, in a limited and briefly reported study, human lymphocytes were incubated with a styrene (99% purity) concentration of 0.03% (about 3 mM) for 72 hours (Linnainmaa *et al,* 1978a and 1978b). Whole blood cultures were used with no exogenous metabolic activation. A statistically significant increase in chromosome breaks was noted, these occurring in 19% of the cells treated with styrene, as compared to 1% of the controls. Interphase analysis of 1000 cells treated with styrene at a similar concentration revealed a statistically significant increase in the number of cells with micronuclei and nuclear bridges (10%, compared with 2% in controls). An increase in aneuploidy was also observed in treated cells (9% compared with 2% in controls).

Overall, although none of the individual *in vitro* chromosome aberration studies is entirely satisfactory, in comparison with current test guidelines, collectively they appear to show that styrene does have the potential to produce chromosome damage *in vitro*.

Sister chromatid (SCE) studies have also been conducted. In a series of studies, styrene was found to induce a significant increase in SCE activity in human lymphocytes or Chinese hamster ovary (CHO) cells when whole blood cultures or erthyrocytes were used as the source of metabolic activation (Norppa *et al,* 1980a; Norppa *et al,*1983b; Norppa and Vainio 1983a; Norppa and Tursi; 1984; Norppa *et al,*1985; Norppa *et al,* 1983a; De Raat 1978; and Chakrabarti *et al,* 1993). However, negative results were obtained when purified human lymphocytes alone or lymphocytes or CHO cells in the presence of induced rat liver S9 (Norppa and Tursi, 1984) were assayed with styrene concentrations of 1-20 mM (Norppa *et al,* 1983a; Norppa and Tursi 1984).

In another study, a dose-responsive and statistically significant increase in the number of SCEs/cell has been reported in an *in vitro* SCE study when human whole lymphocyte cultures were exposed to styrene (99% purity) for 48 hours at concentrations of 0.5 to 5mM (Norppa *et al,* 1980b; and Norppa and Vainio 1983b).

In three briefly reported unscheduled DNA synthesis (UDS) assays, styrene at concentrations of up to 1000 µM was found to be inactive when cultured with human resting lymphocytes (Lopraino 1978; Pero *et al,* 1982; Brunnemann *et al*, 1992). No further details were reported.

DNA binding studies

A study designed to investigate the covalent building of [8-^{14}C] styrene to the DNA of isolated rat hepatocytes *in vitro* is available (Legraverend *et al,* 1984). Freshly isolated hepatocytes from male Wistar rats, either untreated or following a 4-day treatment with phenobarbital, were incubated for 3-5 hours with 1mM styrene. Following incubation, cell nuclei were isolated, lysed, the DNA isolated and the radioactivity measured. Increased levels of radioactivity were measured at 3 and 5 hours post-exposure in the DNA of the hepatocytes of the phenobarbital-treated animals, but not of the untreated animals. The nature of the bound material and of the binding itself was not characterised.

Summary of *in vitro* studies

The overall picture presented by the *in vitro* assay results available is that at least in some test systems (including Ames tests and *in vitro* chromosome aberration studies in mammalian cells), styrene does posses some genotoxic potential *in vitro*. Metabolic activation (presumably to styrene oxide) is required for this activity.

4.1.2.7.2 Studies in Drosophila

The mutagenicity of styrene has been investigated in Drosophila melanogaster, by measuring the number of recessive lethal mutations using the Base procedure (Donner *et al,* 1979). A statistically significant increase in the frequency of recessive lethal mutations was observed. This effect was found to be enhanced by phenobarbitone pre-treatment of Drosophila. Styrene produced no effect on induction of sex chromosome non-disjunction in Drosophila, although the induction of such changes was produced by the positive control used (Pentila *et al,* 1980).

4.1.2.7.3 In vivo studies in mammalian systems

Chromosome aberration studies

Inhalation

In a chromosome aberration study, Sprague-Dawley rats (4 per sex per concentration) were exposed to 0, 600 or 1000 ppm (0, 2550 or 4250 mg/m^3) styrene for 6 hours/day, 5 days/week for 12 months (Sinha *et al* 1983). Negative controls were exposed to filtered air. There were no positive controls. For the first 38 days of exposure, the highest concentration used was 1200 ppm but as "anorexia" was observed, the exposure level was reduced to 1000 ppm for the remainder of the study. Following the end of exposure on the last day, animals were treated with colchicine and sacrificed 4 hours later. Only the cells with 42 centromeres were scored. Approximately 80 metaphases were scored per animal in bone marrow samples. Bone marrow toxicity was not observed, there being no decrease in mitotic index in treated groups when compared with controls. One male animal preparation per styrene exposure level was of insufficient quality and therefore could not be included in the analysis. There were no chromosome breaks or gaps in styrene-exposed animals. There was an irregular pattern of higher frequencies of chromatid gaps in the exposed animals but it was not dose-related and is considered not to be of toxicological significance. Although this study had some limitations, in that it used an unusual design and failed to incorporate a positive control, the result was negative.

In a poorly reported chromosome aberration study, male Wistar rats were exposed to 0 (sham-exposed) or 300 ppm (1275 mg/m^3) styrene for six hours/days, five days/week for 2-11 weeks (Meretoja *et al*, 1978). Animals were sacrificed weekly and bone marrow samples taken. The number of animals from which samples were taken for chromosome analysis varied with the sacrifice time; from week 8 there were 4 or 5 per group. There were 100 metaphases examined per animal. No evidence of toxicity was presented and no positive control data were reported. Analysis of the samples from animals sacrificed weekly revealed no evidence of increased chromosome aberrations in samples from weeks 2-8. However, there was an increase (from 2-3% in controls to 7-9%) in chromosome aberrations (breaks) from 9 weeks onwards. The breaks were stated to be almost exclusively chromosome-type breaks. Metaphases from all the animals exposed to styrene for 11 weeks also exhibited polyploidy; the incidence varied from 1-7%. No polyploidy was noted in controls at any stage during the study. Statistical analyses were not presented with any of the results. The results from this study display an odd pattern, in that there was no response from weeks 2 to 8, but an apparent increase in chromosome aberrations and polyploidy in weeks 9-11. It is difficult to conceive of a rational biological explanation for this time-course. Because of this and the generally poor reporting, the study findings, looked at in isolation, are of suspect reliability.

In another study, groups of 6 rats were exposed to 0 150, 500 or 1000 ppm (0, 640, 2120 or 4250 mg/m^3) styrene for 6 hours/day, 5 days/week for 4 weeks (Preston and Abernethy, 1993). Blood samples were taken at 1, 2, 3 and 4 weeks after the initiation of exposure and 4 weeks after the end of exposure. Peripheral lymphocytes (a minimum of 25 cells per animal) were analysed for chromosome aberrations. Negative results were obtained. A

"positive control" of ethylene oxide was used but no positive response was elicited, calling into question the sensitivity of the protocol used. The failure of the positive control and the analysis of an inappropriately small cell number definitely limit the importance of this negative study.

In a series of studies, female B6C3F$_1$ mice and Fischer F344 rats were exposed via whole-body inhalation to 0, 125, 250 or 500 ppm (0, 530, 1060 or 2120 mg/m^3) styrene for 6 hours/day for 14 days (Kligerman *et al*, 1992; Kligerman *et al*, 1993). There were no positive controls in the study and toxic signs were not reported. A toxicity study in the same strain of mice is available in which deaths occurred at 250 ppm during a 2-week period of repeated exposure. Hence it would appear that the dose levels used were sufficiently high. Spleen and lungs were removed from mice, and peripheral blood from rats, one day after the last exposure and cells cultured. Cells were cultured from spleen from 6 mice, and metaphase analysis carried out at approximately 45 hours. Lung cells (type of cell not specified) were cultured from each of the other 8 animals in each group and analysed for chromosome aberrations at 48 hours. Where possible, 100 first division metaphases were scored per animal and samples from 5-6 animals were analysed per treatment group per endpoint. There were no statistically significant increases in chromosome aberrations in splenocytes or lung cells in mice or in peripheral lymphocytes in rat. Overall, this was a clearly negative result, although the study lacked a positive control.

A further study involved inhalation exposure of Chinese hamsters to styrene at 300 ppm (1275 mg/m^3) for 6 hours/day (three hours on the last day), daily for 4 days or 5 days/week for 3 weeks (Norppa *et al*¸ 1980a). There were 3 or 4 animals per group. The number of metaphases analysed was 100-150 per animal in treated groups and negative controls. The study was reported only in brief, with no toxicity or positive control data being presented. Nonetheless, there was no increase in chromosomal aberrations noted in the bone marrow of styrene-exposed animals.

In summary, there are major limitations to most of the six inhalation chromosome aberration studies available. However, none of the tests that can be considered for evaluation revealed a positive result. A single strudy where a positive result was reported (Meretoja et al., 1978) has major flaws. Overall, the balance of evidence from the six inhalation studies available suggests that styrene does not express chromosome damaging activity in animals exposed by inhalation up to concentrations causing systemic toxicity.

Oral

A bone marrow chromosome aberration study conducted in the CD-1 mouse is available (Loprieno *et al*¸ 1978). Groups of 9 control or 4 treated animals were given oral gavage doses of 0, 500 or 1000 mg/kg styrene and sacrificed 24 hours later. At the higher dose, 2 animals died and only 88 cells in total were available for analysis. The number of cells analysed in the control group and 500 mg/kg group was 800 and 400 respectively. No statistically significant increases in chromosome aberrations occurred. Contrary to styrene, styrene oxide administered in the same study at a dose of 50 and 500 mg/kg was positive. Mitomycin C was used as a positive control and caused a strong induction of chromosome aberrations. Hence this study, using high dose levels of styrene, gave a clearly negative result.

In another study groups of 10 CD-1 male mice were treated with daily oral gavage doses of styrene and bone marrow samples were analysed for chromosome aberrations 24 hours after the last exposure (Sbrana *et al*, 1983). The doses were 500 mg/kg for 4 days (6 treated animals with 3 controls) or 200 mg/kg for 70 days (7 treated animals with 3 controls). One hundred cells were scored per animal. There was no effect on mitotic index but in previous

work, a single dose of 1000 mg/kg was lethal. There were no increases in chromosome aberrations due to styrene treatment; a positive response was observed with the positive control substance cyclophoshamide. Therefore, this study, again using high doses, also yielded a clearly negative result.

Intraperitoneal

Chromosome aberrations were analysed in 100 metaphases per animal from bone marrow samples taken 16 hours after intraperitoneal administration of styrene at 0 or 50-1000 mg/kg to groups of 3-4 male C57BL/6 mice (Sharief *et al*, 1986). Cyclophosphamide was used as a positive control. The mitotic index was reduced at 750 and 1000 mg/kg, indicating cytotoxicity at the higher doses, and samples from only one animal were available at 1000 mg/kg as 1 out of 4 animals and 3 out of 4 animals died at 750 and 1000 mg/kg respectively. There were no significant increases in the number of cells with chromosome aberrations in any of the styrene-treated groups, indicating a clearly negative result.

Overall the general pattern of results from the significant number of *in vivo* chromosome aberration studies that have been conducted in the rat, hamster and the mouse, following single or repeated exposure to styrene up to concentrations and/or doses causing systemic toxicity, via the inhalation, oral and intraperitoneal routes in the tissues examined (bone marrow, peripheral lymphocytes and slpenocytes) is negative.

Micronucleus assays

Inhalation

A micronucleus assay was conducted at the same time as the chromosome aberration study described above (Kligerman *et al,* 1992 and Kligerman *et al,* 1993). F344 rats (5 per concentration) and $B6C3F_1$ mice (6 per concentration) were exposed to concentrations of 0, 125, 250 or 500 ppm (0, 530, 1060 or 2120 mg/m^3) for 14 days. Mouse peripheral lymphocytes and splenocytes were cultured. Micronuclei were also analysed in rat and mouse whole blood smears and rat bone marrow smears. Splenocyte cultures for the micronucleus assay were harvested at 51 hours. In general, 1000-2000 polychromatic erythrocytes and 1000 splenocytes were scored per animal. The P/N ratios in mouse or rat peripheral blood or in rat bone marrow were not altered by styrene treatment, indicating no cytotoxicity. No styrene-related increases in micronuclei were observed in any of the tissues examined in either species.

In another briefly reported mouse micronucleus assay 4 groups of 7 male $B6C3F_1$ mice were exposed whole-body to styrene vapour (99% purity) at a concentration of 50 ppm (210 mg/m^3) for 8 hours (Leavens *et al*, 1997). Control animals were exposed to air (negative) or 200 ppm benzene (positive). Exposure to styrene did not affect the frequency of MN-PCE in the bone marrow, assessed at 24 hours post-exposure (frequencies were increased in the benzene positive controls), but was cytotoxic as shown by a significant decrease in the total number of spleen cells.

Groups of 6-7 male NMRI mice were exposed, whole-body, to 0, 750, or 1500 mg/m^3 (0, 173 or 345 ppm) styrene vapour 6 hours/day for 1, 3, 7 or 21 days (Vodicka *et al*, 2001). Animals were killed shortly after exposure on the last day of each exposure interval and bone marrow was removed for analysis of micronuclei (MN). No positive control group was included in the study. Two thousand polychromatic erythrocytes (PCEs) were scored from each animal. There was a statistically significant increase in the frequency of MN at 1500 mg/m^3 on day 7

only (10.5/1000 PCEs); on day 1,3 and 21, values were approximately equal to controls. At 750 mg/m^3 there were no statistically significant differences from controls. These results contrast with those of a subsequent study by the same authors (Englehardt *et al*, 2003), and the authors themselves question the validity of the observed increase in MN frequency only at one time-point as it does not appear plausible that styrene exposure should lead to micronucleus induction at 7 days but not after one or three days, given its known rapid metabolism and distribution.

In a subsequent study replicating the conditions of earlier work by Vodicka *et al* (2001) and conforming to current OECD and GLP guidelines, groups of 5 male NMRI mice were exposed, whole-body, to 0, 750 or 1500 mg/m^3 (0, 173 or 345 ppm) styrene vapour 6 hours/day for up to 21 days (Englehardt *et al*, 2003 – authors include Vodicka). Animals were killed shortly after exposure on the last day of each exposure interval and bone marrow was removed for analysis of micronuclei independently by two laboratories. Two thousand polychromatic erythrocytes (PCEs) were scored from each animal. Although direct evidence for target organ exposure (e.g. reduced P/N ratio) was lacking, general toxicity was evidenced by the general poor condition and subsequent death of 7/35 animals at 1500 mg/m^3 (345 ppm) within the first 6 days. Reduced bodyweight gain (details not provided) was seen amongst both groups of styrene-exposed animals during the first 3 days of exposure and this persisted until the end of the study.On completion of 1, 3, 7, 14 and 21 days of exposure there was no statistically significant increase in micronucleated PCEs at either exposure level. Positive (intraperitoneally injected animals with 20 mg/kg cyclophosphamide) and negative controls showed the expected responses. These results are in contrast with the previous study by Vodicka *et al*, 2001 that showed an occasional (only on day 7) statistically significant increase in micronucleus formation at 1500 mg/m^3. On balance, this inter-laboratory and more recent study shows that styrene does not produce bone marrow clastogenicity in mice exposed by inhalation at exposure levels (up to 1500 mg/m^3) that produced evident systemic toxicity.

Intraperitoneal

A micronucleus study in the Porton rat and the LACA Swiss mouse is available (Simula and Priestly, 1992). Results were obtained from 4 – 10 male animals per treatment. Single intraperitoneal injections of vehicle, styrene or cyclophosphamide (positive control) were administered. Styrene doses were 300-3000 mg/kg and 150-600 mg/kg in rats and mice respectively. Bone marrow samples were taken at 30, 48 and 72 hours. At the highest dose, 40% of the rats and 50% of the mice died. The only effect on the P/N ratio was a slight treatment-related decrease in the rat; however, this indicates that the substance had reached the target tissue. There was no evidence of styrene-induced micronucleus formation in the rat. In mice, the only statistically significant increase (to 4.2 micronucleated polychromatic cells per 1000, against 1.3 in controls) in micronuclei occurred with the highest dose at 48 hours in mice. Therefore in this assay, robust negative results were obtained in the rat. In the mouse, the single instance of a statistically significant higher incidence of micronuclei arose at a dose level at which 50% of the animals died. Whether or not this signifies an effect genuinely attributable to styrene is unclear; and even if so, secondary consequences of severe general toxicity may have been a complicating factor. Hence this result, viewed in isolation, should not be seen as convincing evidence of a mutagenic effect of styrene in mice.

This study also investigated the potential effects of styrene on sperm morphology. Groups of 4-10 male Porton rats and LACA Swiss mice were given intraperitoneal injections of 0, 250, 500, 1000 or 2000 and 50, 100, 200 or 400 mg/kg/day styrene, respectively, on 5 consecutive days. Additional groups of rats and mice given intraperitoneally 5, 10, 20 or 40 mg/kg cyclophosphamide, were used as positive control animals. Mice and rats were then

sacrificed at 3, 5, 7 and 5, 8, 11 weeks, respectively, from the start of dosing to explore potential effects on the early spermatid, primary spermatocyte and spermatogonial stages of spermatogenesis. Head-shape morphology was examined for 1000 sperms per animal. At the highest dose of styrene (2000 mg/kg/day) and at the highest dose of cyclophosphamide (40 mg/kg/day), 90% and 80% of the rats died, respectively. A small, but statistically significant increase in the proportion of abnormal sperm was observed in rats (1.6 fold) at 1000 mg/kg/day at 5 and 8 weeks but not at 11 weeks from the start of dosing, and in mice (2.8 fold) at the top dose of 400 mg/kg/day at 5 and 7 weeks but not at 3 weeks from the start of dosing. Cyclophosphamide, the positive control substance, induced a greater increase in the proportion of abnormal sperm than styrene in both rats (4-11.5 fold at 20 mg/kg/day) and mice (3.5-5 fold at 40 mg/kg/day), although the effect was not of the expected magnitude for a positive control.

Overall, a weak increase in abnormal sperm (without convincing evidence of a dose response) was seen in this i.p study in mice at 400 mg/kg/day and in rats at 1000 mg/kg/day. However, it is noted that the interpretation of the response in rat was confounded by the severe toxicity observed in these animals and that the mouse result is inconsistent with that of another study in which no abnormalities in sperm morphology were seen in animals exposed by inhalation and intraperitoneally up to 300 ppm and 700 mg/kg/day styrene respectively (Salomaa *et al*., 1985; see section 4.1.2.6.1). This endpoint is also non-specific for genotoxic effects and no evidence of mutagenic potential is indicated by the weak responses seen.

In another study bone marrow micronucleus test, groups of four C57BL/6 male mice were treated by the intraperitoneal route with 250, 500, 1000 or 1500 mg/kg styrene (Norppa, 1981). There was a negative control group of 13 animals. Animals were sacrificed at 30 hours only and analysis of 2000 polychromatic cells per animal carried out. No positive control results were reported. The highest dose was lethal to 2 animals and the P/N ratio was significantly decreased at 1000 and 1500 mg/kg. The frequency of polychromatic cells with micronuclei was higher, but not in a dose-responsive manner, in treated animals compared with controls, being 4.9, 8.4, 6.8, 9.9 and 7.3 per 1000 cells at 0, 250, 500, 1000 and 1500 mg/kg, respectively. The increase above control levels was statistically significant only at 250 and 1000 mg/kg. Hence some evidence for a positive result in mouse emerged from this study, although the magnitude of the apparent increases seen was small and the pattern of results was unusual, with no dose-related upward trend. The significance of the findings should be judged in the context of the overall database on the mutagenic potential of styrene. For example it should be noted that the positive result obtained at 250 mg/kg is inconsistent with those obtained via the inhalation route at concentrations up to 1500 mg/m^3 (equivalent to approximately 600 mg/kg).

Negative results were obtained in another bone marrow micronucleus test, in the Chinese hamster, at the single intraperitoneal dose of 1000 mg/kg, but the study was limited by the use of only one sacrifice time (30 hours), no positive control and only 2 animals in the negative control group (with 4 animals per treated group). Under the same experimental conditions styrene oxide (250 mg/kg) also revealed a negative result (Penttila *et al*, 1980).

Overall, six micronucleus assays have been performed in the mouse and two in the rat, using the inhalation or intraperitoneal exposure routes. The balance of evidence suggests that styrene does not express genotoxic potential in this test up to concentration and/or doses causing systemic toxicity. In the three instances where micronucleus frequency in styrene-treated mice was higher than in the negative control group, other features of the results (discussed above) indicate that these findings should not be regarded as convincing evidence of mutagenicity *in vivo*.

Unscheduled DNA Synthesis (UDS)

Inhalation

In a recent *in vivo* mouse liver unscheduled DNA synthesis (UDS) assay (Clay, 2004), conducted to GLP and OECD guidelines, groups of 6 female CD-1 mice were exposed whole body via inhalation to 0, 125 or 250 ppm styrene monomer (99.9 % purity) for 6 hours. The highest concentration level was selected based on the findings of a pre-test in which groups of 5 female mice were administered a single 6-hour inhalation exposure of 160, 250, 500 or 1000 ppm styrene. The concentration level of 250 ppm was determined as the maximum tolerated dose based on observations of slight to moderate glycogen deposition and focal/multifocal mixed inflammatory cell infiltration of the liver (the key organ of the UDS analysis) at 250 ppm, portal inflammation and multifocal single cell necrosis of the liver at 500 ppm, mortality and clinical signs of toxicity at 1000 ppm. Female animals alone were used in the study since malignant neoplasms were seen in females and not males in the available mouse inhalation chronic toxicity/carcinogenicity study. An additional group of 6 female mice was administered a single oral gavage dose of the positive control substance, N-nitrosodimethylamine (N-DMA) at 10 mg/kg. Following exposure, 3 animals/concentration group were sacrificed at 2 and 16 hours post-exposure for isolation and preparation of hepatocytes. The freshly isolated hepatocytes were then cultured in the presence of tritiated thymidine and subsequently examined for UDS following autoradiography.

No treatment-related signs of toxicity were observed in the test animals. Hepatocyte viability ranged from 38 to 74 %. Mean viability of liver cells from the negative control animals was 62% (69% for the 2 hour sampling time and 55% for the 16 hour sampling time). Although the viability of liver cells from one single negative control animal was relatively low (38%), for the remaining 5 control animals, the viability of liver cells ranged from 63% to 74%. Furthermore, since this animal did not show a UDS response significantly different from the other negative control animals, it was considered acceptable. Styrene caused no significant increases in mean net nuclear grain count or in mean cell percentage in repair compared to the control animals. The positive control substance produced appropriate responses. Overall, styrene monomer did not induce DNA repair as measured by unscheduled DNA synthesis in hepatocytes of female CD-1 mice exposed *in vivo* via the inhalation route.

Sister Chromatid Exchange (SCE)

Inhalation

SCEs were analysed in samples of bone marrow, alveolar macrophages and regenerating liver cells from 15 control and 4 styrene-treated male BDF_1 mice per group (Conner *et al,* 1979, 1980a and 1980b). Animals were exposed to styrene (99% purity) at concentrations of 0, 104, 387, 591 or 922 ppm (440 – 3900 mg/m^3) for 6 hours/day for up to 4 days. Duplicate groups of hepatectomised mice were included to study the effect of styrene on more rapidly diving cells in the regenerating liver. Statistically significant and dose-related increases above the controls of 0.3 to 4-fold in the numbers of SCEs/cell were observed at styrene exposure concentrations of 387 ppm and above in all 3 tissue types after exposure for 4 days. Smaller effects were measured after exposure for 2 days and there was only a marginal effect in regenerating liver cells after exposure for 1 day. It is surprising that there is no effect in bone marrow after exposure for 1 day and a generally higher effect after 4 days than after 2 days. SCEs usually represent an immediate response to the exposure of the target cells measured in mitoses after two rounds of replication. Styrene is rapidly distributed and activated and does not accumulate after repeated dosing. Therefore, despite the

positive result in this study, the effects are not completely plausible and may be – at least in part – not directly related to stryrene-induced DNA damage.

In an earlier study by the same authors (Conner *et al*, 1979) using a similar protocol, groups of 4 BDF_1 male mice were exposed to 0, 50 or 70 ppm styrene, via whole-body exposure, for 5 hours/day for 4 days. A 50% increase in the number of SCEs/cell was reported in the alveolar macrophage and regenerating liver cells of mice exposed to 50 ppm, compared with non-exposed controls. At 70 ppm a very low yield of mitotic cells limited the interpretation of the results generated for these two tissues. In bone marrow, the number of SCEs/cell was also higher, but only slightly, in styrene-treated animals compared with controls (3.1 in controls compared with 3.3 and 3.4 at 50 and 70 ppm, respectively).

As part of a study in the F344 rat and $B6C3F_1$ mouse which has been described above, animals were exposed to 125 - 500 ppm (530 – 2125 mg/m^3) styrene for 14 days (Kligerman *et al*, 1992; Kligerman *et al*, 1993). There were 6-8 mice per group and 5 rats per group. SCEs were analysed in cultures of peripheral blood, spleen and lungs removed from mice one day after the last exposure. The type(s) of lung cell analysed was not specified. Peripheral lymphocytes from rats were also cultured and analysed for SCEs. Cells were cultured for 48 hours. Where possible, 50 second division metaphases were scored. There was a small, concentration-related increase in SCEs in the various tissues, which was statistically significant at 125 ppm (mouse) or 250 ppm (rat) and above.

In another study, groups of F344 rats were exposed to 0, 150, 500 or 1000 ppm (0, 640, 2125 or 4250 mg/m^3) styrene for 4 weeks in a SCE assay in peripheral lymphocytes conducted at the same time as the chromosome aberration analysis described above (Preston and Abernethy 1993). There were no increases in SCEs in styrene-treated animals at any concentration or sampling time. A positive control (ethylene oxide) group showed an appropriate response at all sampling times.

Intraperitoneal

In an SCE assay, groups (size not specified) of Porton rats were treated with 0 or 375-3000 mg/kg styrene and groups of LACA Swiss mice received doses of 0 or 75-450 mg/kg styrene intraperitoneally (Simula and Priestly, 1992). Sacrifice times were 48 hours (rats) and 34 hours (mouse). The positive control was cyclophosphamide. SCEs were determined in splenocytes cultured for 65 (rat) and 43 (mouse) hours. Results were available for 5 animals per treatment apart from the highest dose where 4 mice and 1 rat were available; twenty metaphases were analysed per animal. There was no evidence of spleen cytotoxicity resulting from styrene treatment. In rats, a statistically significant and dose-related increase in SCE (133-204% of controls) occurred at 750 and 1500 mg/kg. At 3000 mg/kg 4 out of 5 animals died. In mice, 1/5 animals died at the highest dose (450 mg/kg) and there was a “slight” statistically significant increase in SCE (117% of controls) at this level only. The positive result obtained in this study indicates that styrene induced persistent DNA alterations which enhanced SCE formation when cells were stimulated to proliferate in vitro. However, the statistical analysis seems to be inappropriate because the analysis is obviously not based on the individual animal means. Furthermore, the effect in mice was very small and limited to a toxic dose. In rats, a slight increase in the SCE frequency was measured after exposure to 750 mg/kg and a clear effect was only seen at the highest analysable dose (1500 mg/kg). On the whole, the test indicates a weak SCE-inducing effect of styrene at a near lethal dose in mice and a comparatively stronger effect in rats.

A study in male C57BL/6 mice included the analysis of SCEs in bone marrow 24 hours after an intraperitoneal injection of 0 or 50 - 1000 mg/kg styrene (Sharief *et al,* 1986). Thirty metaphases per animal were assessed in the study. No effect was determined on replication

index. The only statistically significant increase in SCE was in the one animal examined at 1000 mg/kg; the results for the other lower dose groups of 2-4 animals were negative. A positive control produced an appropriate response. Given that only one animal was available for examination at the top dose, the reliability of the apparently positive finding is questionable.

Overall, the general pattern of SCE results in the wide range of tissues examined (lymphocytes, splenocytes, bone marrow, alveolar macrophages, regenerating liver cells) from both the rat and the mouse following inhalation or intraperitoneal exposure to styrene has been positive. However, it is important to note that in most cases concomitant chromosome aberration and/or micronucleus assays involving the same animals and in some cases the same tissues were carried out and that negative results were obtained for these established indicators of chromosome damage. Therefore it is clear that the observations of styrene-induced increases in the frequency of SCE do not correspond to the appearance of clear evidence of chromosome damage in standard regulatory tests. SCEs are the result of intrachromosomal recombination. Their formation is strongly enhanced in cells after induction of DNA damage that interferes with DNA replication. The SCE test has been very popular in the past because it is more sensitive than the classical chromosome aberration test for detecting most DNA-damaging chemicals and easier to perform. However, since the biological significance of induced SCEs remains obscure, the interest in this test has decreased such that during the last ten years no new SCE studies have been published for styrene.

DNA strand breaks

An experiment was conducted to determine whether or not inhalation styrene exposure produced any DNA strand breaks under alkaline conditions in a single-cell gel assay (comet assay) in peripheral lymphocytes (Kligerman *et al*, 1993). Five F344 rats per group were exposed to 0 or 125 – 500 ppm styrene 6 hours per day for 14 consecutive days. The comet assay was performed one day after the last exposure and about 50 peripheral lymphocyte cells were analysed per animal. There were no statistically significant increases in alkali-labile DNA strand breaks.

Groups of 6-7 male NMRI mice were exposed, whole-body, to 0, 750, or 1500 mg/m^3 styrene vapour 6 hours/day for 1, 3, 7 or 21 days (Vodicka *et al*, 2001). Animals were killed shortly after exposure on the last day of each exposure interval and peripheral lymphocytes, bone marrow, and hepatocytes were removed for analysis of DNA strand breaks using the comet assay, and using endonuclease III to detect oxidised pyrimidines and apurinic/apyridimic sites. No significant increase in single-strand breaks was observed in bone marrow, but there was an increase in endonuclease-sensitive sites after 21 days of exposure to styrene only. However, this effect was not concentration-related and possibly due to a low concurrent control. In lymphocytes an isolated positive response was found after 7 days and endonuclease III posttreatment did not show any enhancing effect. In hepatocytes, there were no significant changes in strand breaks or endonuclease-sensitive sites. Overall, the findings reported in this study are equivocal and might just be due to high assay variability.

Alkali-labile single strand breaks (SSB) were measured in DNA from liver, lung, kidney, testis and brain in male NMRI mice sacrificed 1, 4 and 24 hours after a single intraperitoneal dose of 0 to 1000 mg/kg styrene (Solveig-Walles and Orsen, 1983). The DNA unwinding technique with alkaline elution was used. The level of alkali-labile SSB was increased in a dose-dependent manner in the kidney 4 hours after administration of styrene. Increases were also noted in the other organs at 1 hour and, apart from liver, at 24 hours. These increases were highest at 24 hours after administration. The percentages of SSB induced in

the testis were estimated from the figures and calculated from the transformed data to be increased from approximately 8% in controls to 11% in the treated animals which indicates a very marginal effect. Furthermore, no information on dose response for testicular SSB was provided, although the time course data indicate an increase. Overall, since this study is poorly reported with no statistical analysis and indication of variability in control values and with data only presented diagrammatically as a log-transformation, it cannot be considered to be evidence of a positive response. Furthermore this endpoint may reflect general toxicity rather than genotoxicity as, considering the rapid metabolism and distribution of styrene, it is not plausible that some effects were seen 24 hours after the end of the exposure but not at an earlier time point. This might indicate that the delayed effects were not due to styrene-induced DNA-SSB but a result of indirect effects (e.g. repair activity, cytotoxicity).

The comet assay was used to study the DNA-damaging effect of styrene in female mice after a single intraperitoneal injection at a dose up to 500 mg/kg (Vaghef *et al*., 1998). DNA strand breaks (increase in tail moment) occurred in lymphocytes, liver, bone marrow and kidneys 4 hours after treatment. Smaller effects were measured 16 hours after exposure. In comparison to styrene, styrene oxide was more potent in inducing DNA effects in the comet assay under these test conditions.

In summary, there are indications from these studies that styrene at high doses leads to DNA strand breakage in various tissues.

DNA binding studies

Covalent binding of ^{3}H-styrene to DNA was assessed in liver (both species) and lungs (rats only) after groups of B6C3F1 mice and F344 rats inhaled styrene in a closed chamber for up to 9 hours (Lutz 1992; Cantoreggi and Lutz, 1993). Initial concentrations of styrene in the chamber were about 275 - 465 ppm (1169 –1972 mg/m^3). No binding was seen up to the limit of detection in rat liver. The binding index (mol adduct/mol DNA/mmol chemical/kg) was 0.07 in the lungs of 2/4 rats, and essentially zero in the other two. In mouse liver, binding indices were 0.05-0.18. The significance of this finding in relation to the mutagenic potential of styrene is unknown.

The formation of DNA adducts in rat and mouse liver, whole lung and isolated lung cells (of different types) following inhalation exposure to styrene has been investigated (Boogaard *et al*., 2000). Twelve male Sprague-Dawley rats and 30 male CD1 mice were exposed nose-only to 160 ppm (680 mg/m^3) ^{14}C-styrene vapour for 6 hours. Groups of 2 rats and 5 mice exposed to air served as controls. Immediately following exposure (time = 0 hours) and again at 42 hours post-exposure 6 rats and 15 mice per test group were sacrificed for DNA adduct analysis. Adducts were detected in all the cell types examined. In the rat liver the major DNA adduct present was that of N7-guanine-styrene oxide. In the mouse liver the adduct levels were qualitatively and quantitatively different to those observed in the rat. Total adduct levels were significantly higher in the mouse liver than in the rat and in the mouse several unidentified adducts (adducts designated X, XI, I) were more prominent than the N7-guanine-styrene oxide adduct. In both the rat and mouse total lung analysis levels of adducts were low (in the range of 1:10^8) and in most instances were below the limit of detection of the assay. However the most prominent adduct detected in each species was the N7-guanine-styrene oxide adduct, present at similar levels in both species. Quantitatively, there was no species or tissue specificity of DNA binding that could be correlated with tumour induction in the lung of mice. In the mouse, adducts VIII and X were also present. The isolated lung cells analysed were macrophages, Type II enriched (Type II) cells and Type II depleted (non-Type II) cells in the rat and Clara and non-Clara cells in the mouse. In the rat macrophages no statistically significant levels of adducts were detected for either species. In the Type II cells of the rat the major adduct detected was the N7-guanine-styrene oxide

adduct. No adducts were detected in the non-Type II cells of the rat lung. In the mouse Clara and non-Clara cells the major adduct was adduct I.

Groups of 6-7 male NMRI mice were exposed, whole-body, to 0, 750, or 1500 mg/m^3 styrene vapour 6 hours/day for 1, 3, 7 or 21 days (Vodicka *et al*, 2001). Animals were killed shortly after exposure on the last day of each exposure interval and DNA was extracted from lungs for analysis of styrene oxide 7-guanine and 1-adenine adducts using HPLC; both adducts were found on days 1, 3, 7 and 21 at both exposure levels, and the levels detected increased with increasing duration of exposure. Low levels of adduct formation, close to detection limits, were also observed on occasion amongst controls. Styrene oxide 7-guanine adducts were more prevalent than 1-adenine adducts.

Frozen liver samples from groups of 5 male and 5 female Sprague-Dawley rats that had previously been exposed via inhalation to 0, 50, 200, 500 or 1000 ppm (0, 210, 840, 2100 or 4200 mg/m^3) styrene vapour for 6 hours/day, 5 days/week for 2 years in a carcinogenicity study (see Cruzan et al, 1998) were used for analysis of deoxyguanosyl-O^6-styrene oxide adducts by ^{32}P-postlabelling (Otteneder *et al*, 2002). In addition, DNA adducts were analysed from the lungs of groups of 5 female Sprague-Dawley rats exposed to 0 or 500 ppm for 2 weeks and in lung samples of 5 female CD1 mice exposed to 0, 40 or 160 ppm (0, 170 or 680 mg/m^3) styrene for 2 weeks. Increased adduct levels (around 8-9 O^6-guanyl adducts/10^7 nucleotides) were only seen in the 2-year rat liver samples of the highest concentration group (1000 ppm). No adducts were detected in the lungs of rats and mice exposed to styrene for 2 weeks. Therefore, on the basis of the data obtained in mice, it is unlikely that styrene-induced adduct formation correlates with tumour formation in the lungs. The authors concluded that the particular susceptibility to tumour formation of the mouse lung might have been due to cell-type specific toxicity and tumour promotion.

In another study, groups of male NMRI mice received doses of ^{14}C-styrene of up to 510 mg/kg by the intraperitoneal route (Nordqvist *et al*, 1985). There were no negative controls. Binding of radioactivity to DNA was observed in the liver, brain and lungs; the adduct chromatographed at the same position as N7-(hydroxyphenylethyl)-guanine. Covalent binding of styrene to N7-guanine was also detected, albeit at very low levels (lower than in the other organs investigated), in the testis. Increases in this DNA adduct of up to 31 nmol/g DNA and in binding to protein and haemoglobin were seen with increasing dose. The levels of DNA adducts induced in this study are below those associated with mutagenic effects and styrene adducts at N7 guanine are unstable and generally give rise to apurinic sites which are repaired.

Groups of 7 NMRI mice were administered a range of doses up to 4.35 mmol/kg (510 mg/kg) of styrene (98% purity) via the intraperitoneal route (Pauwells *et al*, 1996). Three hours post-administration blood, liver, lung and spleen were collected and analysed for styrene-specific N-7 and O^6-guanine DNA adducts and N-terminal valine adducts, using the ^{32}P post-labelling assay and the GC-MS Edman degradation technique, respectively. For all adducts a clear dose-related increase in adduct formation was observed in all tissues examined.

In summary, these DNA binding studies indicate that various organs from rats and mice exposed to styrene by intraperitoneal injection or inhalation are actually exposed to styrene in an active form. This exposure results in an interaction with DNA leading to various covalently bound DNA adducts. The mutagenic potential of these adducts is not completely understood. However, a comprehensive study indicated that the styrene-induced DNA adduct level determined in tissue homogenates from animals on a 2-year carcinogenicity study did not reflect the species- and organ-specific tumour induction in experimental animals (Otteneder *et al*., 2002).

Summary of in vivo studies in experimental animals

Styrene has been exhaustively studied in clastogenicity studies up to dose levels producing severe toxicity in some cases. There is no convincing evidence of styrene clastogenicity in experimental animals when the quality of the studies and the plausibility of the test results are considered. Equivocal results were obtained after exposure to high doses causing lethality. However, overall, negative results were obtained from *in vivo* chromosome aberration and micronucleus studies in the rat, hamster and the mouse following single or repeated exposures to styrene up to concentrations and/or doses causing systemic toxicity, via the inhalation, oral and intraperitoneal route in the tissues examined (bone marrow, peripheral lymphocytes, splenocytes and whole blood). Furthermore, a recently published micronucleus test in bone marrow cells of mice conforming to the current OECD guideline was clearly negative.

The general pattern of SCE results in wide range of tissues examined (lymphocytes, spleenocytes, bone marrow, alveolar macrophages, regenerating liver cells) from both the rat and the mouse following inhalation or i.p exposure to styrene has been positive. However, it is important to note that in most cases concomitant chromosome aberration and/or micronucleus assays involving the same animals and in some cases the same tissues were carried out and that negative results were obtained for these indicators of chromosome damage. Therefore, this clearly reduces the significance of the SCE findings in relation to mutagenicity.

The binding of styrene metabolites to DNA was very low and did not indicate any specificity for the target tissue (mouse lung). Induction of alkali-labile single-strand breaks has also been produced *in vivo* in rats and mice exposed to styrene. Again the significance of these findings is unclear, given the repeated failure of styrene to demonstrate mutagenic activity in standard clastogenicity assays.

In contrast to the weakly positive findings in indicator tests detecting SCEs, DNA strand breaks and DNA adducts, an in vivo UDS test performed in accordance with international guidelines did not reveal a genotoxic effect of styrene in mouse liver.

Overall, based on standard regulatory tests, there is no convincing evidence that styrene possesses significant mutagenic/clastogenic potential *in vivo* from the available data in experimental animals.

Studies in humans

A large number of studies have been conducted to investigate mutation frequency, chromosome aberrations, micronucleus formation, SCEs, DNA adducts and DNA strand breaks in the peripheral lymphocytes of workers exposed to styrene in the glass reinforced plastics (GRP) industry. Variations in the way data were presented makes a meaningful assessment of some studies very difficult. In addition, studies of this type cannot reliably link exposure to a chemical with genotoxicity; control groups are difficult to match adequately, there is significant heterogeneity in any population and pre-exposure data are not available for individual chemical-exposed workers.

Mutation assays

The frequency of *hprt* mutants were determined in the peripheral lymphocyte samples from 46 workers exposed to styrene in the GRP industry and in a group of controls matched for age, sex and smoking habits (Tates *et al*, 1994). The exposed workers were subdivided into

groups of 22 and 24 because blood samples for these groups were collected one week apart. Information on medication being taken, health status, prior exposure to radiation and alcohol consumption was obtained by questioning each subject. Exposure to styrene was determined from personal monitoring. The concentrations of styrene were 17 ppm (range 0-142 ppm) 8-hour TWA in the pooled exposed group. Co-exposure to dichloromethane, an established genotoxin, could not be ruled out. The poor condition of 18 control samples reduced the number of available control results to 5. As a consequence, no statistical analysis was available when comparing exposed and control results. In the control group, the mean mutation frequency was 8.6×10^{-6} (standard deviation 1.2×10^{-6}); results in each exposed subgroup were not significantly different from each other, the pooled mean frequency being 14.3×10^{-6} (standard deviation 15.7×10^{-6}). Some samples with very low cloning efficiencies were used which is an invalid approach. This probably accounts for the high variability in response. Values quoted in the literature for background levels of *hprt* mutants in the general population are for example 6×10^{-6} in human lymphocytes (Cole and Skopek, 1994). The range of values in the exposed groups was very large and the higher values were found among the smoking sub-population. No assessment of the intra-individual variation in the study population was provided, and no correlation with duration of exposure was found. For these reasons and on the basis of potential co-exposure to dichloromethane, no conclusions on the effects of styrene alone can be drawn from this study.

As part of a study investigating several endpoints the *hprt* mutation frequency in the peripheral lymphocytes of 9 lamination workers has been measured at 6 time points over a 3 year period (Vodicka *et al*, 1995, and cited in Lambert *et al*, 1995). A group of 9 lamination workers (7 females and 2 males, average age 43 years) who had been employed for several years (average 6.7 years) was included in the study. A non-styrene exposed group of 7 administrative workers (4 females; 3 males) of similar age (average 41 years) and employed in the same factory served as referents. A control group of 8 laboratory workers (all female; average age 49 years) was also assessed at one sampling timepoint. All individuals studied were non-smokers. Blood samples were collected from the individuals for analysis on four different occasions over an eight-month period; sample 1 – immediately prior to vacation (July 1993), sample II – immediately after a 2 week vacation (August 1993), sample III – 1 month after the return to work (September 1993), sample IV – 7 months after study onset (February 1994). Peripheral T-lymphocytes were analysed for *hprt* mutation frequency. Air sampling was conducted twice, firstly three days prior to sample I and secondly on the day of sample IV using personal monitors over a 6-hour shift. Blood styrene levels and mandelic acid levels in urine were also measured to confirm exposure.

At sample time I, styrene mean exposure level of 28 ppm (SD 17 ppm) was measured (although unclear, these are probably TWAs). This level however decreased over the duration of the study, to 22 ($\pm$18) ppm at sample time IV. Blood styrene and urinary mandelic acid levels confirmed this downward trend in styrene exposure. No details regarding possible co-exposures to other substances are reported. Mutation frequencies (MF) did not differ significantly in laminators when compared against the factory referents at any time point. Mean MF in laminators varied from 15.3 ($\pm$5.9)/10^6 to 20.6 ($\pm$25.9)/10^6 (sample time I) and in factory controls from 12.8 ($\pm$7.2)/10^6 to 18 ($\pm$13)/10^6. MF was measured in the laboratory controls only at sample time IV. The MF was lower in the laboratory controls than in the styrene-exposed workers (11.8 ($\pm$6.8)/10^6 compared to 18 ($\pm$5)/10^6). This difference was statistically significant only at the fourt sample time. In view of the large individual differences in MF (8-fold) presumably reflecting the relatively small numbers of cells plated for mutant frequency and evidence of clonal expansion in the highest MF individuals, the isolated difference seen at one single sampling time is not convincing.

A subsequent report providing details of further analysis of this group of lamination workers has been published (Vodicka *et al*, 1999). This report included data on the original 9 lamination workers and 7 factory administrator controls. Techniques employed were the

same as in the previous report but included two extra sampling times giving the following sample time points; I – now taken in December 1992; II – I day prior to the summer vacation in July 1993; III – first day after the two weeks vacation; IV – after an additional month at work in September 1993; V – in February 1994; and VI – in March 1995. The populations studied at sample time V and VI included 13 laminators (8 of which were included throughout the study) and 13 laboratory controls (apparently new to the study). Four of the laminators and 5 of the laboratory controls were smokers. Personal air monitoring (in the breathing zone for 6 hours) was performed four times on 11 laminators (1 day prior to sample I; 3 days prior to sample II; and on the same days as samples V and VI). Urine and blood samples were analysed for mandelic acid and styrene respectively as further markers for exposure. As before, *hprt* MF was measured in the peripheral T-lymphocytes from blood samples taken at the above times.

Mean airborne exposure concentrations were ~37 ±22 ppm at sample time I reducing to ~16 ±11 ppm at sample time VI possibly due to improvements in exposure conditions. The levels of urinary mandelic acid and blood styrene found in the laminator workers did not correlate with the airborne exposures and showed a high degree of inter-individual variability (which again did not correlate with airborne exposure). Data were reported for the *hprt* MF at sample time VI. In laminators the mean ± SD MF ($22.3 \pm 25/10^6$) was statistically significant different from that ($4.2 \pm 46.4/10^6$) determined in laboratory controls. This difference was seen when comparisons were made within the smoker and non-smoker subgroups. A correlation was also observed between age and MF (increasing with age) and number of years worked and MF. Although these data may represent an effect of styrene exposure of *hprt* MF in the lymphocytes of these workers, it is recognised by the authors of the study that such measurements can be influenced by a wide range of factors such as different types of T-cells with different life-spans, background exposures and passive smoking. Furthermore, it is noted that the increase in *hprt* MF did not correlate with internal or external measures of styrene exposure. Also, air levels declined over time whereas the MF remained constant. There was a large interindividual variation in MF and many individuals had very low cloning efficiencies which might have invalidated the assay. Age resulted to affect the MF but the age distribution was similar in both groups. Thus, although the results of this study may indicate an effect on *hprt* MF in the T-lymphocytes of these lamination workers, they are indicative rather than conclusive evidence of a mutagenic effect of styrene.

These two previous reports were further analysed by Vodicka *et al* (2002a) in terms of cumulative exposures. Unfortunately, this analysis is lacking in detail which makes it difficult to evaluate. The MF data were analysed in relation to duration of exposure and also in relation to an arbitrary factor (exposure coefficient) which took account of exposure levels over the period of employment and the total exposure time. Both parameters were claimed to be correlated with MF, but individual data were not presented. Hprt MF is known to be a function of age, but it is not clear from the paper (particularly as control control data were not presented) how this was taken into account. Also, correlation with the exposure coefficient appears to be largely influenced by the high MF value of one single older worker. Overall, there is no convincing evidence that these mutations were induced as a function of duration of exposure independent of age. Also, there is no information on whether smoking and other potential genotoxic exposures were accounted for.

A further study (Vodicka et al., 2003) analysed the results obtained in 1995 and 1999 reporting no statistically significant difference in *hprt* MF between the exposed and the control group.

In summary, a series of studies conducted by Vodicka and colleagues over a number of years on the same population reported a negative response in the first series (Vodicka *et al*., 1995), a positive response in the follow-up study (Vodicka *et al*., 1999), and finally a lack of

statistical difference between exposed workers and control individuals over the whole study population (Vodicka et al., 2003).

The frequency of glycophorin A (GPA) mutants was determined in peripheral blood samples from 47 workers exposed to styrene in the GRP industry (drawn from 10 factories of varying size) manufacturing small boats, large containers and pipes (Bigbee *et al,* 1996). Work tasks included carpentry, molding, cleaning, waxing, laminating and resin mixing. A group of 47 non-exposed controls employed in research institutes and university was also sampled. Controls and workers were matched for age, sex and smoking habits and were questioned regarding health status and employment history. Workers and controls reporting a history of radiotherapy or chemotherapy were not included in the study. GPA variant erythrocyte frequencies (V_f), reflecting GPA allele loss (Ø/N), and allele loss duplication (N/N) were determined by flow cytometry. The assay is based on the autosomal GPA locus that codes for the erythroid lineage-specific cell surface sialoglycoprotein responsible for the M/Ns blood group. Exposure to styrene was determined from personal air monitoring. The mean personal exposure concentration of styrene in GRP workers was 37 ppm 8-hour TWA (range 6 to 114 ppm). Styrene exposure in workers was confirmed by the measurement of urinary mandelic and phenyl glyoxylic acid levels.

No statistically significant differences were observed between variant cell frequencies for either ø/N or N/N between the workers and controls. A high degree of variation in the V_f values within the groups was observed ranging from ~2.5 – 53 per million cells for ø/N and 0.4 - 40 for N/N. Also, replicates assessed at the same time showed a two-fold variation. No significant effect overall was seen on GPA ø/N or N/N in a multivariate analysis of covariance in which styrene exposure, gender and smoking status were treated as independent variables and age as a continuous variable. When styrene exposure was divided into low (1 - 84 mg/m^3 and high $\geq$ 85 mg/m^3), then a statistically significant increase in N/N V_f was seen only in women workers for the adjusted geometric mean values. It is noted that one exposed individual with a very low V_f was excluded from the statistical analysis. When this subject was included, no significant difference between exposed and control group was seen. Age was highly associated with an increase in N/N V_f and smoking was found to be a significant confounding factor. Although some statistically significant associations were found in this study between some aspects of styrene exposure and changes in N/N V_f using multivariate analysis, these were isolated findings across a number of tests which assess a highly variable endpoint. Given that smoking was also a significant confounding factor and that the same subjects studied here have previously been assessed for chromosome aberrations, SCEs and MN with negative responses (see Sorsa *et al*., 1991), the significance of the marginal response observed in this study is considered inconclusive.

Overall, there is no convincing evidence that styrene exposure induces mutations in vivo in humans.

Cytogenetic assays

Chromosome aberrations and micronuclei

The frequency of cells with aberrant metaphases or micronuclei (MN) was measured in peripheral lymphocytes of 21 GRP workers and 21 controls matched for sex and for smoking habits (Maki-Paakkanen, 1987). There was no difference between exposed and controls regarding alcohol or drug intake, vaccinations or viral infections according to interview responses. The number of smokers (15) and non-smokers (6) was the same in both groups. Exposure to styrene was on average 23 ppm, range 8 - 63 ppm, 4-hour TWA. One hundred

metaphases per subject were scored for chromosome aberrations and 1000 PCE per individual were analysed for MN. There was no increase in the mean number of metaphases with chromosome aberrations (including and excluding gas) in the exposed group (3.0 ± 0.3%, excluding gaps) compared with controls (3.7 ± 0.5%). Also, no difference between exposed (1.5%) and control groups (1.6%) was found in the percentage of micronucleated cells.

The same research group analysed the frequency of cells with aberrant metaphases or MN in peripheral lymphocytes of 17 GRP workers and 17 controls (Maki-Paakkanen *et al*., 1991). One hundred metaphases per subject were scored for chromosome aberrations and 500 PCE per individual were analysed for MN using the cytocholasin B method. Urinary mandelic acid concentrations were used to calculate air levels in the plant (mean level of 69 ppm). No increase in the frequency of chromosome aberrations excluding gaps was found in the exposed workers (3.1%) compared to controls (3.0%). However, the incidence of chromosome aberrations including gaps was higher in non-smoking workers than in non-smoking controls. No increase in the frequency of MN was found. Since an increase was only found for chromosome aberrations including gaps in non-smokers but not for this group excluding gaps or for the whole group, this cannot be considered sufficient evidence for a clastogenic effect of styrene.

The frequency of metaphases with chromosome aberrations in peripheral lymphocytes from a group of 11 exposed GRP workers (1.27% excluding gaps) was not increased above the frequency in 11 controls of similar age and lifestyle (1.36%, excluding gaps) (Jablonicka *et al*, 1988). One hundred metaphases were examined per person. The GRP group was exposed to an average of 60 ppm but with a range of up to 139 ppm styrene; the type of average and reference period was not specified.

Thiess *et al* (1978 and 1980) found no significant increase in the frequency of metaphases in peripheral lymphocytes with chromosome aberrations (either inclusive or exclusive of gaps) in two studies of 12 and 24 workers exposed to styrene in a polystyrene production plant. Levels of styrene exposure, in air, in the two studies were reported as being 0.2 - 47 ppm and 6 - 58 ppm, respectively (type and reference period not reported). One-hundred metaphases were scored for each individual. The frequency of chromosomal aberrations in exposed workers was reported at 1.9% compared with frequencies of 1.9 and 1.5% in control groups. In both studies controls were matched for age and sex. All subjects were questioned with respect to their smoking habits, alcohol intake, viral diseases, recent vaccinations, x-ray treatment, exposure to known clastogens and medicinal drugs. However no details of the numbers of smokers per group, nor individual data were reported.

Watanabe *et al* (1981) measured styrene exposure levels in two styrene workshops (a fibre reinforced plastics boat factory and a polyester-resin board factory) of 1 - 20 ppm 4-hour TWA. A geometric mean of 6 ppm in the boat factory and a geometric mean of 33 ppm in the resin board factory were measured. In the boat factory 4-hour TWA values of 100 - 200 ppm were found in 4 workers, and in the resin-board factory a 4-hour TWA of 2 - 43 ppm was measured. There were 9 workers at the first workplace and 7 at the second who were exposed to styrene. Age and sex matched controls (group size 5 and 8) were included. About 100 metaphrases were analysed per person. There was no increase in the frequency of metaphases with chromosome aberrations (including gaps) in the peripheral lymphocytes of the exposed workers compared to the controls (mean frequency in control and exposed groups 2.9 and 3.6%). The analysis did not report the frequency of aberrations excluding gaps.

The same researchers analysed chromosome aberrations in lymphocytes from 18 styrene-exposed workers employed in a fibre reinforced plastics boat factory and 6 age-matched controls (Watanabe *et al*, 1983). The personal exposure levels were estimated to be 40 - 50

ppm “TWA” concentrations (no further details were given). There were similar frequencies of metaphases with aberrations (excluding gaps) in the exposed (1.12%) ad control (1.07%) groups.

In another study controls were age-matched to two groups of styrene-exposed workers (Pohlova and Sram, 1985). Polystyrene workers (36 in total) were exposed to 17 - 36 ppm styrene (mean, whole-shift level) and GRP (23) workers were exposed to up to 48 ppm. One hundred metaphases were examined per individual. There were no increases in the frequency of cells with chromosome aberrations (excluding gaps) in the peripheral lymphocytes of styrene workers (exposed groups 1.14%, 2.81%, controls 1.26%, 1.88%).

In a poorly reported but apparently well-conducted study, blood samples were taken from 109 styrene-exposed GRP workers and 54 controls from plastics and other industries to assess chromosome aberrations (Norppa *et al*, 1991 and Sorsa *et al*, 1991). A subgroup of 50 exposed and 37 controls were also assessed for MN, using the cytocholasin B method. No details of the controls (not exposed to styrene) were given but age and smoking were taken into account in this study. The highest exposure levels from personal monitoring were 39 - 43 ppm 8-hour TWA in laminators (range 5 – 182 ppm) and 14 ppm in other exposed workers. The authors reported no increases in the “total number” of chromosome aberrations in peripheral lymphocytes of the exposed workers compared to controls (100 metaphases analysed per individual: mean values from 12 non-exposed plastics workers given as “1.8”, other controls “1.6”, and exposed groups “1.4-2.4”). Increases were reported in older subjects and smokers. Five hundred PCEs were analysed per sample for MN. The percentage of micronucleated cells were 0.7 in laminators and 0.8 in controls, indicating no difference in MN frequency between exposed and unexposed subjects.

Hagmar *et al* (1989) examined the frequency of metaphases with chromosome aberrations in peripheral lymphocytes from 11 glass reinforced plastics workers exposed to a mean styrene concentration of 13 ppm (personal sampling over 60 hours), together with 15 controls. Information was gathered on current medication, smoking habits, viral infections and exposure to ionising radiations. One hundred metaphases were scored per person; the mean frequency of chromosome breaks in the exposed group was 1.2/100 cells compared with 1.5/100 cells in controls. Even when the authors accounted for possible effects of age and smoking by multiple regression analysis, there was no effect of styrene exposure. MN were also scored in 1000 lymphocytes per individual from 20 workers and 22 matched controls. The frequency of MN was not increased when the effect of age and smoking was accounted for by multiple regression analysis.

Chromosome and chromatid breaks were measured in peripheral lymphocytes from 15 workers exposed to an average of 24 ppm styrene (reference period not given) in a glassfibre reinforced plastics factory (Nordenson and Beckman, 1984). A control group of 13 workers (most being salesmen and office clerks with no known styrene exposure) included 3 smokers compared with 4 in the exposed group; age ranges were similar (21 – 61 years in exposed; 26 – 53 in controls). Two hundred metaphases were examined per individual. Twelve exposed workers and 12 controls were also assessed for MN induction. No significant differences in the numbers of breaks were seen between exposed and control groups, although only gaps and breaks were reported in this limited study. A significant increase in the incidence of MN was seen in the exposed group (3.5%) compared to controls (0.8%). However, there were some technical problems with the MN technique with cultures from 4 individuals unsuitable for analysis. Also, other workplace exposures were not accounted for. Overall, no conclusions can be drawn from this study.

The blood from a group of 18 workers in a glass fibre reinforced polyester plant was sampled together with that from 9 controls (office workers at the same factory) of approximately the same age and smoking habits (Hansteen *et al*, 1984). Styrene exposure was measured in

the breathing zone of the workers and the mean concentration was 13 ppm (no further details given). Medical history and alcohol consumption data were obtained. Styrene exposure was confirmed by analysis for the metabolites, mandelic acid and phenylglyoxylic acid in end of shift urine samples. One hundred metaphases from peripheral lymphocytes per person were analysed in whole blood cultures. There were no differences in the mean number of chromosome aberrations (possibly only breaks were scored) per 100 cells in control (1.7) and exposed subjects (1.2). A statistically significant increase in the number of gaps was observed but no biological significance can be attributed to this finding.

An increase in chromosome aberrations has been reported in the peripheral blood lymphocytes of a sub-group of 23 male workers exposed to 20 - 320 ppm styrene (levels determined by personal air sampling, over 3 to 8 hours) in a fibre-reinforced plastic boat building facility, but not in another group of 23 workers exposed to 0.46 – 28 ppm styrene (Artuso *et al*, 1995). Control subjects (51) from the same geographical area and with no history of occupational exposure to genotoxic agents were matched by age, sex ad smoking habits. Other potential confounding factors such as X-ray exposure, working history, medical history, medical intake, use of alcohol, drugs and smoking habits were evaluated. An average of 150 metaphases per subject were scored. Blood samples were sent to two different laboratories for cytogenetic analysis, one laboratory receiving 26 samples (13 each of exposed and control) and the other 79 samples (42 control, 37 exposed). The percentage of smokers in the high exposure group was greater than that in the other groups (60% compared with 45% in controls and 30% in the low exposure group), although the mean number of cigarettes smoked per day was highest in the low exposure group (16.6 cigarettes/day in the high exposure group compared with 23.6 in the low exposure group and 17.3 in the control group). There were more people undergoing diagnostic X-rays in controls (90%) compared to low (48%) and high (48%) exposure groups. An increase in the number of aberrations (excluding gaps) per 100 metaphases was observed in the higher exposure group and was statistically significant at 4.00 compared with 2.13 in controls and 2.75 in the low exposure group. The number of aberrations, in both workers and controls, reported by the 2 scoring laboratories was stated to be inconsistent, with higher scores being awarded by laboratory 1 compared with laboratory 2. These differences were accounted for in the statistical analyses. Overall, given the potential for confounding because of smoking and the scoring differences between readers and laboratories encountered in this study, no conclusions can be drawn.

A statistically significant increase in the frequency of metaphases with chromosome aberrations has been reported in blood lymphocytes of a group of 18 male workers manufacturing reinforced plastics (Anwar and Shamy 1995). A non-exposed control group (administrators from the same factory) of 18 males matched by age and sex were also tested simultaneously. All of the subjects were non-smokers and had been employed in the factory for 10-22 years. No details of styrene levels in air in the workplace were reported. Styrene exposure was determined by analysis of urinary mandelic acid and thioether levels, both of which were statistically significantly higher (2-fold greater) in exposed workers compared with controls. The number of metaphases analysed for aberrations per individual and the incubation time were not reported. No details of any consideration of other confounders are discussed. The percentage frequency of chromosome aberrations in exposed individuals was, with gaps, 6.06 and, without gaps, 4.00, compared with 3.44 and 1.44 in controls. No correlation with exposure was found. The frequency of MN (cytocholasin B method) was also measured, but no difference between exposed (6.55/1000) and controls (6.00/1000) was seen. Overall, given the lack of information on whether there were confounding exposures, no conclusions can be drawn from this report.

A well-conducted cytogenetic study has reported a higher frequency of chromosome aberrations in the metaphases of the peripheral blood lymphocytes from a subgroup of 11 workers exposed to 27 – 104 ppm styrene (personal air sampling, no other details such as

reference period given) compared to matched (for age, sex and smoking habits) controls from 2 factories using polyester resins (Tomanin *et al,* 1992). No increase was observed in another subgroup of 7 exposed subjects employed in the same factory but exposed to lower concentrations of styrene (5 – 23 ppm). Other potential confounding factors such as X-ray exposure and medication intake were evaluated but no details were reported. An average of 100 metaphases per subject were scored. The increase in the higher exposure group of 11 subjects was statistically significant for percentage of aberrant metaphases (3.02, standard deviation 2.28) compared with that in controls (0.82, standard deviation 0.75). This was also true for the total numbers of aberrations. There was no correlation between the incidence of aberrant metaphases and the exposure levels. MN were also scored in 17 workers and 17 controls using the cytocholasin B method, but no difference between the exposed and unexposed groups was seen.

A small group of lamination workers (10) was studied together with 5 controls (Meretoja *et al*, 1977). The controls were apparently not matched but the ages of the controls were within the range of ages of the exposed individuals. No exposure data were available but mandelic acid was measured in the urine of workers. At the time of the study, the selected subjects stated that they were healthy and had not been suffering from recent viral infection or had exposure to any agent known to have clastogenic activity. Chromosome aberrations were scored in lymphocytes from exposed and unexposed individuals (100 metaphases per person) but only “chromosome-type breaks” were reported. The incidence was 11-26% in laminators and 3% or below in controls. MN were also scored (no details on the methodology employed provided), but no difference between the exposed (0.88%) and unexposed groups (0.08%) was seen. No statistics were presented. There was no correlation between urinary mandelic acid levels and the frequency of cells with chromosomal aberrations or MN. Also, smoking and other workplace exposures (e.g. peroxides used for curing in the lamination process) were not accounted for. The same researchers repeated their experiment in the same GRP workers and controls at a later date, with similar results (Meretogja *et al,* 1978). Overall, given that important confounding factors were not taken into account, no conclusions can be drawn from these two studies.

Another study analysed chromosome aberrations in the peripheral lymphocytes from only a small number (6) of exposed GRP and non-exposed age and sex matched control workers (Hogstedt *et al*, 1979). The workplace exposure concentration for styrene was 12 - 95 ppm TWA (time period not specified) from personal sampling. Groups were questioned about factors such as smoking habit and drug intake and no occupational exposure to known genotoxic agents was discovered. There were 3 smokers in each group. Two hundred metaphases were examined per person. The sum of chromosome aberrations (not defined) including gaps was 10.8 per 100 cells compared to 5.2 in controls and chromosome breaks (excluding gaps) were also statistically significantly increased (6.9 per 100 cells, 2.5 in controls). The frequency of aberrant metaphases was not given and tabulated results were not presented, making the results difficult to interpret. There was no correlation between exposure levels and the number of breaks. Overall, in view of the poor reporting and the low number of subjects investigated, no firm conclusions can be drawn.

Samples of lymphocytes were taken from groups of styrene-exposed workers at a styrene manufacturing plant, a polystyrene plant and 3 GRP processing plants in a study of chromosome aberrations (Fleig and Thiess, 1978). Atmospheric styrene levels were measured in different parts of each exposure area, however results were not reported. One hundred metaphases were analysed per person. Results from groups exposed to styrene were compared with those in control groups. No details on the matching of controls and exposure groups wee reported. Data on age, alcohol consumption, smoking habits, previous occupational exposures, recent viral infections, vaccinations and medication including radiography examination was obtained for all workers. Only 5 exposed workers’ samples were analysed from styrene manufacture because the exposure concentrations were low,

but only urinary metabolite exposure data were reported. There were cytogenetics results available for 12 workers in polystyrene manufacture and 14 in the GRP industry. An increased mean frequency of aberrant cells, excluding gaps, of 5.3% compared with 2.1% in controls, was observed in GRP workers. However no significant increase in the number of aberrations was observed in either the styrene manufacturing (1.6%) or the polyester manufacturing (1.9%) groups.

The frequency of chromosome aberrations was analysed in peripheral lymphocytes taken from 36 styrene-exposed workers at a boat-building facility and from 37 unexposed factory personnel matched for age and sex (Andersson *et al*., 1980). Airborne styrene exposure measurements were obtained from breathing zone sampling on 6 occasions over 6 years. Average daily exposures (8-hour TWA) were calculated. The exposed group was then divided in high- and low-exposure subgroups (mean styrene concentrations of 277 and 32 ppm respectively). An increase in the number of aberrations excluding gaps was found in the exposed group (7.9%) compared to controls (3.2%), but there was no difference between the high- and low-exposure subgroups. Rings and dicentrics, which are rare aberrations, were found in both control and treated groups (0.4% and 1% respectively). This casts some doubts on the scoring and on the causative agent for these lesions since styrene does not induce these *in vitro*. Also, the authors report the necessity to culture for long time to get enough metaphases; this must be a reflection on poor culture conditions, and therefore, the possibility of introducing artefacts cannot be excluded. Furthermore, other exposures (e.g. methylethyl-ketone peroxide, a known genotoxin), which could have confounded the response, were present in the workplace. Overall, no conclusions can be drawn from this study.

Another study focussed on a group of 42 workers employed in 9 different GRP plants (Camurri *et al*, 1983 and 1984). Styrene air levels were stated to be 7 - 95 ppm (reference period not given). Twenty-two unexposed controls were matched for sex, age and smoking habits. None of the exposed or unexposed subjects had had recent viral infections, vaccinations or reported exposure to clastogenic agents. Mean frequencies of metaphases with chromosome aberrations, determined from 100 metaphases per subject, were statistically significantly increased to 23-44% in the exposed group compared with 5-9% in controls. Most aberrations seen were of the chromatid type. It is not clear if these values included or excluded gaps, and the frequencies reported in both exposed and control subjects are very high; no similar data could be found in any other study including those that report similar styrene exposure levels. There was no correlation between exposure levels and the frequencies of aberrant metaphases. Overall, the reliability of these findings is uncertain.

Tates *et al,* (1994) studied 46 workers using unsaturated polyester resins in the production of containers and exposed to styrene at an 8-hour TWA concentration of 17 ppm (range 0 - 142 ppm). Co-exposure to dichloromethane, an established genotoxin, could not be ruled out. A control group of 22 workers matched for age, sex and smoking habits, employed in a machine building factory and not exposed to any other known genotoxin, was also analysed. Workers were questioned regarding health status, prior exposure to ionising radiation, drug use, smoking and alcohol consumption. Peripheral whole blood samples were collected from workers and controls for analysis of chromosome aberrations and MN. No details on the methodology employed for the MN analysis were provided. The results were not presented in the usual way and the number of metaphases per individual was not reported. Data on numbers of aberrations (including gaps) and MN rather than numbers of aberrant or micronucleated cells, were reported. In the exposed group, there were 2.9% (SD=1.3%) chromosome aberrations including gaps compared with 0.9% (SD=0.8%) in controls. This increase was statistically significant. An increase in the MN frequency was also seen in the exposed workers (35/1000) compared to controls (14.3/1000). In this study, results were

reported with insufficient detail, and on the basis of potential co-exposure to dichloromethane, no conclusions on the effects of styrene alone can be drawn.

A small group of 30 lamination workers (aged 22 - 58 years and employed for 1 - 30 years) was studied together with 2 controls (Dolmierski *et al* 1983). No information regarding the matching of controls or possible confounders (ie smoking habits, medication, previous exposures, etc) were reported. No exposure measurement data were presented but it is stated that exposures were below 23 ppm. An incidence of chromosomal aberrations in peripheral blood lymphocytes of the exposed workers of 6.8% was reported. No value for controls was reported and no statistics were presented. There was no correlation between duration of employment and the frequency of cells with chromosomal aberrations. Overall, given the limitations in the reporting, no conclusions can be drawn from this report.

A nested case-referent study conducted in myeloid leukaemia patients previously exposed to styrene is available (Kolstad *et al*, 1996). The aim of the study was to determine if there is any relationship between myeloid leukaemia and clonal chromosome aberrations in styrene-exposed workers. For the period 1964 to 1970, 36,525 workers employed in the production of reinforced plastics and exposed to styrene and 14,254 workers employed in the same industry but not exposed to styrene, were identified. There was no matching between these groups with respect to factors such as gender, age, smoking habits, and ethnic origin, although the population as a whole was drawn from a homogenous workforce with respect to social and economic factors. Thirty-four workers developed myeloid leukaemia. Of these, 9 had chronic myeloid leukaemia (CML) and 25 had acute myeloid leukaemia (AML). Bone marrow chromosome aberration (CA) analysis data were available for 7/9 (78%) CML patients and for 12/24 (48%) AML patients. None of these patients had had a previous malignancy and the chromosome analyses were not conducted secondary to chemotherapy. Blood samples for CA analysis were also taken from 57 age-matched referents, selected from the “population at risk” (exposed group) at the time of the patient diagnosis. No measure of individual exposure was available. Proxy measures of “high” or “low” exposures were determined from information provided by the companies.

Of the 6 out of 7 patients with CML and CA data who had been exposed to styrene, 5 were found to have an abnormal karyotype (the 9:22 translocation; the “Philadelphia” chromosome), whilst the 1 unexposed CML patient had a normal karyotype. In the styrene workers a 2.5-fold increase in the risk of myeloid leukaemia with clonal chromosome aberrations (OR 2.5 95% CI=0.2-25.0) was determined compared with unexposed workers No other significant cytogenetic patterns were observed. Although the results are suggestive of a possible relationship between chromosome aberrations, development of leukaemia and exposure to styrene, no firm conclusions can be drawn given the low number of patients in this analysis, the lack of specific exposure data and the incomplete case ascertainment. Furthermore, the presence of CAs in cancer cells is normally associated with the tumourigenic process, and hence, it is difficult to ascertain whether there is any link with exposure to styrene.

Metaphases from the peripheral lymphocytes of 3 groups of workers employed in a lamination factory in Czechoslovakia along with those from 2 groups of control unexposed workers were assessed for chromosomal aberrations (100 metaphases/individual) (Popler *et al*, 1989). Levels of styrene exposure were determined via personal samplers and confirmed by analysis of mandelate levels in urine. The first group of workers consisted of 13 women, average age 45 years with an average occupational exposure of 11.5 years. Group 2 consisted of 12 women and 3 men, average age 40 years with an average occupational exposure of about 8 years (this group included 7 individuals of group 1) and an average styrene exposure over a workshift of >60 ppm. Group 3 consisted of 17 women, average age 46 years with an average occupational exposure of about 7 years and an average styrene exposure over a workshift of <2 ppm. Individuals were questioned about potential

confounding factors including their recent medical histories, smoking habits, previous radiographic or chemotherapeutic treatment. Blood samples were taken from these groups in April 1984 and November 1986. Controls groups (design technicians) were sampled in November 1984 (9 women and 12 men; average age about 33 years) and February 1987 (9 women and 8 men; average age 37.6 years). There were slightly more smokers in the exposed groups (6/13 group 1; 13/15 group 2; 5/17 group 3) compared to control (12/21 and 3/18) groups. The incidences of chromosomal aberrations in group 1 (3.54%) and group 2 (3.27%) workers were statistically significantly increased compared with controls (2.00% and 1.65% respectively). The incidence of aberrations in group 3 workers was comparable with controls. Although these changes were statistically significant, the biological significance is uncertain given the small differences seen. Furthermore, the difference in average age and proportion of smokers between the groups increases the difficulty in interpretation.

The frequency of chromosome aberrations was analysed in peripheral lymphocytes taken from 44 styrene-exposed workers at a hand lamination plant and from 19 unexposed office workers at the same factory matched for age and sex (Somorovska *et al*., 1999). Styrene exposure was measured by workplace monitoring (details not given), blood styrene levels and alveolar sampling immediately after leaving the workplace. The exposed group was then divided in 3 subgroups of low, medium and high exposure (mean 8-hour TWA of 6, 13 and 46 ppm). Current exposure to other chemicals was excluded by chemical analysis, and smoking was accounted for. All 3 exposed groups had elevated frequencies of aberrant metaphases (3.27% and 2.50% and 3.75% in low, medium and high exposure groups respectively) compared to controls (1.37%), although no dose-reponse was detected.

In another study, the frequency of chromosome aberrations was analysed in peripheral lymphocytes taken from 10 workers exposed to styrene and 21 healthy controls, not matched for sex or smoking (Biro et al., 2002). No information on exposure was provided in the study report. An increase in chromosome aberrations was found in the exposed workers (3.8%) compared to controls (1.8%). However, the increase was not statistically significant and was within the historical control levels. Furthermore, the much higher incidence of smokers in the exposed group confounds this result, and therefore, no conclusions can be drawn from this poorly conducted study.

No exposure-related increase in micronucleated peripheral lymphocytes was found in a study of 50 of 54 male styrene-exposed workers and 41 unexposed control individuals (Karakaya *et al*, 1997). Workers were exposed to styrene for a mean duration of 9.9 years ($\pm$7.1 years) during the lamination of furniture with polyester resin. Exposure levels ranging between 20-300 ppm were measured in the breathing zone of the workers using a stationary sampler (mean exposure measured during lamination was 152 ppm, and 10 minutes post lamination was 30.3 ppm). Workers and controls were matched for age (average age of both 27 years) and smoking habits (both groups contained 70% smokers). All subjects were question with respect to their occupational, medical, family and dietary histories. Peripheral lymphocyte samples were incubated for 96 hours at 37^{o}C and 1000 cells per individual were analysed for micronuclei. The mean percentage of micronucleated cells was 1.98 $\pm$ 0.50 in laminators (2.18 in non-smokers and 1.91 smokers) compared with 2.09 $\pm$ 0.35 in controls (1.82 in non-smokers and 2.20 in smokers).

No exposure-related increase in the frequency of micronucleated lymphocytes was found in a study of 25 exposed workers (17 males and 8 females) employed in 5 areas of a styrene production plant compared with 25 ‘unexposed’ control individuals employed in the same plant (Holz *et al*, 1995). Workers were employed for an average of 18 years (1 to 34 years) and were of an average age of 39 years. Workers and controls were matched for age and sex. Individuals were questioned regarding their smoking habits, diet, alcohol consumption, use of medication and possible hydrocarbon exposure outside the workplace. No significant differences between workers and controls with respect to any of these parameters were

reported. Smoking was also determined by measurement of plasma cotinine levels. Thirteen exposed workers and 17 unexposed workers were smokers. Airborne exposure levels were determined using passive personal monitoring over 1 hour in the exposure areas and 3 hours in the control areas. In the exposure areas styrene levels ranged from <0.01 - 0.83 ppm. Other hydrocarbons present were: 0.08 - 0.53 ppm ethylbenzene, <0.02 - 1.11 ppm benzene, 0.01 - 0.78 ppm toluene and <0.01 - 0.02 ppm xylenes. Examination of company records revealed that previous exposures were “far higher” than those measured (no details of how much greater were reported). Worker exposure was also determined by biological monitoring of exhaled breath and urinary metabolites both before and after an 8 hour working shift. Peripheral lymphocytes samples were incubated for 72 hours at 37^{o}C and 2000 cells per individual were analysed for MN by the cytocholasin B method. In addition, slides were stained with CREST antibodies to determine the frequency of kinetochore positive MN which are indicative of chromosome loss. The mean percentage of micronucleated cells was 1.9 $\pm$ 0.78 in exposed workers (2.03 in non-smokers and 1.84 smokers) compared with 1.87 $\pm$ 0.71 in controls (2.15 in non-smokers and 1.61 in smokers). However, a statistically significant, although small, difference between exposed (15/1000) and unexposed workers (12/1000) in the proportion of MN, which were kinetochore positive, was found. It is considered that the change in distribution of kinetochore positive versus kinetochore negative MN without a concomitant increase in the MN frequency is difficult to rationalise as it implies that a decrease in chromosome breakage was accompanied by an increase in chromosome loss. The authors suggested that the effect might have been due to benzene (possible aneugen) rather than styrene.

The frequency of MN in peripheral blood lymphocytes assessed by the cytocholasin B method did not vary with increasing styrene exposure (by linear regression) among a group of 48 workers exposed to a mean 8-hour TWA of 15 ppm (range 0.2 - 56 ppm) in a reinforced plastic boat manufacturing facility (Yager *et al*, 1993). Information was obtained on age, sex, smoking habits, alcohol consumption, drug intake, viruses and vaccinations. Four hundred cells were analysed per sample. The mean frequency of micronucleated cells per thousand cells was 8.9. No unexposed controls were incorporated in this study.

A group of 49 GRP workers were exposed to a mean styrene concentration of 7 ppm (range 0.5 - 26 ppm, no time specified) and MN measured by the cytocholasin B method in lymphocytes cultured from blood samples (van Hummelen *et al*, 1994). Results for samples from 23 controls were obtained for comparison. The average age of the control group was similar and number of smokers/ex-smokers less in the control group. Information on diet and medication was also obtained. There was no difference in the frequency of MN between exposed and control groups (3.50 and 4.75 micronucleated cells per thousand cells in control and exposed non-smoking groups) and MN incidence did not correlate with styrene concentration.

The mean frequency of MN measured in the peripheral blood lymphocytes of 38 workers employed in the manufacture of polyester resins boats and exposed to a mean 8-hour TWA styrene concentration of 13 ppm (range of 1 - 36 ppm) was stated to be increased when compared with 20 controls (Hogstedt *et al*, 1983). The control subjects were unexposed individuals employed in a paper factory and were matched for age and gender only. Information on viral infections, medication, alcohol use and smoking was available for all subjects. Two techniques were used for lymphocyte preparation, one in which the cytoplasm was preserved and the other using hypotonic treatment. Using the first method, in exposed workers there were 5.9 MN per thousand cells and in controls the value was 3.6 when the long harvest time (96h) was used; this difference was statistically significant. (Frequency of micronucleated cells was not given). It was stated that there was a significant effect of styrene exposure even when confounding factors such as smoking and age were eliminated in a multivariate analysis. In contrast, there was no difference in MN frequency between exposed (3.9/1000) and unexposed groups (3.0/1000) for preserved cytoplasm cells when

shorter harvest time (72h) was used. Also, MN in hypotonically treated cells (2000 lymphocytes scored) were not significantly increased in the exposed workers (4.3/1000) compared to controls (3.7/1000). No correlation between MN frequency and exposure levels was found. The interpretation of these findings is confounded by differences in the methodologies used and differences between the variants. In particular, the finding of increased levels of MN at 96h but not at 72h raises some doubts whether this was a spurious result. The earlier (72h) cultures would have cells which had undergone 1-3 divisions and therefore, if chromosome damage is induced *in vivo*, it should be expressed in these cultures also. Furthermore, other potential workplace exposures (e.g. hydroquinone, methylene chloride, various peroxides, dimethyl analine) typically encountered in polyester resin manufacture were not accounted for. Overall, no conclusions can be drawn from this study.

Brenner *et al* (1991) examined lymphocytes for MN in peripheral blood samples taken from 14 male GRP workers and 9 controls (5 male and 4 female library workers). Geometric mean TWA (time not specified) styrene concentration was 11 ppm (range 1 - 44 ppm). There were a number of females in the control group but not in the exposed group and patterns of medication, smoking and education differed significantly. The number of cells scored per sample was 1000. A statistically significant increase in the number of MN per thousand cells was observed (10.3 in exposed worker samples and 6.5 in controls). (Frequency of micronucleated cells was not given). There was no dose-response with current or cumulative exposure but a measure of exposure derived from the most frequent task in the preceding 4 months did lead to dose-response relationship. Given the obvious differences in the background characteristics of control and exposed groups relating to factors other than styrene exposure, which could influence the results, no conclusions can be drawn from this study. Furthermore, the method for analysing the MN frequency used BrdUrd to determine whether the cells had divided, but introduced potential interaction between BrdUrd and styrene.

Laffon *et al* (2002) analysed the frequency of MN by the cytocholasin B method in peripheral blood samples taken from 14 GRP workers employed for more than 7 years and from 30 controls recruited from university staff. A number of potential confounding factors (age, sex, smoking habits, diet, viral infections, medication) were accounted for in the statistical analysis. However, contribution of organic peroxides (typically encountered in GRP manufacture) to workplace exposure could not be excluded. A significant increase in the frequency of micronucleated cells was found in the exposed group (24.63/1000) compared to controls (13.91/1000). Frequencies of 23.19/1000 and 25.31/1000 respectively were determined for the subgroup of workers employed 7 – 15 years and for those employed >15 years. However, it cannot be excluded that the difference seen between the two exposed groups was due to the age difference rather than to styrene exposure. Overall, a positive result was obtained in this study, but it may not be attributable to styrene exposure because of the potential for other confounding factors to have contributed to the increased incidence of micronuclei observed.

Teixeira *et al* (2004) evaluated if individual polymorphisms in xenobiotic metabolising enzymes related to the metabolic fate of styrene (CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1) could modulate the levels of MN (determined by the cytocholasin B method) in peripheral blood samples taken from 28 GRP workers (employed for a mean of 12 years) and from 28 controls working mainly in office jobs. A number of potential confounding factors (age, sex, smoking and alcohol habits, diet, medication and X-ray exposure) were determined by the administration of a questionnaire. Personal air sampling was performed in the breathing zone for representative working periods. A mean 8-hr TWA of 27 ppm (range 2 – 91 ppm) styrene was determined. Toluene and acetone were also found to be present in the workplace although at levels less than 1% of the styrene concentration. The urinary metabolites of styrene, namely MA and PGA were also measured in samples collected

before the start of the work shift. The mean level of MA + PGA in the exposed population was 401 (range 47 – 1490) mg/g creatinine. A significant correlation between styrene concentration in air and urinary MA + PGA levels was found ($r = 0.72$, $p<0.001$). No statistically significant difference in MN levels between the exposed workers (3.68/1000) and the control group (2.82/1000) was found. Individuals of the low exposure group (MA + PGA < 400 mg/g creatinine) with GSTM1 null genotype had significantly higher levels of MN when compared with GSTM1 non-null individuals. However, this was not observed in the high exposure group (MA + PGA > 400 mg/g creatinine).

In summary, 30 independent publications reporting analysis of clastogenicity in styrene exposed workers are available. These include 23 analyses of chromosome aberrations and 16 of micronuclei. Many of these studies are considered to be inconclusive due either to insufficient detail, possibility of confounding exposures or technical deficiencies such as inadequate harvest time, insufficient cells or individuals. However, of the more robust studies, 8 chromosome aberrations studies are clearly negative and only 3 (Tomanin et al., 1992; Anwar and Shamy, 1995; Somorovska *et al*., 1999) demonstrate weak positive responses. In none of the positive investigations was the response dose related (against personal monitoring data) and in two studies the concurrent assessment of micronuclei, using the sensitive cytocholasin B method, was negative. It is difficult to rationalise why positive responses were found in 4 studies but not the others. These were apparently well-conducted, and the exposures were moderate (from 6 up to 104 ppm) but not excessively high compared to other negative studies (up to 139 ppm).

Ten micronuclei studies were clearly negative, 2 gave a positive response whilst the remainder were inconclusive. In the study reporting a weak positive response (Brenner *et al*., 1991) the possibility of other confounding exposures in the workplace was not addressed. Five studies using the more reliable cytocholasin B method were negative, including one where an increase in chromosome aberrations was reported. However, one well-conducted study using cytocholasin B indicated a positive response (Laffon *et al*., 2002).

Overall, given the extensive number of negative studies reported and the lack of dose-response relationships, the 3 chromosome aberration studies and the 2 micronuclei studies considered as providing evidence of a weak positive response, on balance, do not present conclusive evidence that styrene can cause chromosome breakage in humans.

Sister chromatid exchanges

In the study of Holz *et al* (1995), as detailed above, no exposure-related increases in the number of SCEs/cell (lymphocytes) were observed in 25 styrene exposed workers in a styrene production plant compared with 25 controls. Styrene exposures were measured as ranging from 0.01 to 0.83 ppm. The number of SCEs/cell in exposed individuals was 9.27 (9.38 in smokers and 9.04 in non-smokers) compared with 9.24 in controls (9.67 in smokers and 8.87 in non-smokers).

No exposure-related increases in the number of SCEs/cell were observed in 70 styrene-exposed GRP workers compared with 31 controls (Norppa *et al*, 1991 and Sorsa *et al*, 1991). No details of the controls (not exposed to styrene) were given but age and smoking were taken into account in this study. Styrene levels were up to 39 - 43 ppm 8-hour TWA in the laminators subgroup and 14 ppm in other workers. Worker exposure to styrene was confirmed by measurement of urinary mandelic acid levels (4.3 mmol/l in exposed workers and 1.7 mmol/1 in controls). Fifty metaphases from peripheral lymphocytes samples were analysed per person for SCEs. The number of SCEs/cell in exposed workers was 6.9 - 7.7 in this study.

Meretoja *et al* (1978), examined peripheral lymphocyte samples from 10 lamination workers and 4 controls and found no increases in the number of SCEs/cell (numbers of SCEs/cell were 4.2 - 7.3); no styrene exposure data were given. The controls were apparently not matched but the ages of the controls were within the range of ages of the exposed individuals. Fifteen to twenty metaphases were scored per person.

There were no increases in the number of SCEs/cell in the blood lymphocytes of 21 styrene-exposed workers employed in the reinforced plastics industry relative to 21 unexposed controls matched for sex and smoking habits (Maki-Paakkanen, 1987). Exposure to styrene was on average 23 ppm, range 8 - 63 ppm, 4-hour TWA. No differences were found in vaccination, alcohol consumption or medication histories in the groups. A possible 50 metaphases were scored per sample. The numbers of SCEs/cell were 5.3 - 13.9.

The same research group analysed the frequency of cells with SCEs in peripheral lymphocytes of 17 GRP workers and 17 controls (Maki-Paakkanen *et al*., 1991). Urinary mandelic acid concentrations were used to calculate air levels in the plant (mean level of 69 ppm, 8-hour TWA). No increase in the number of SCEs/cell was found in the exposed workers compared to controls.

Watanabe *et al*, (1981 and 1983) found that when compared with age and sex matched controls there was no increases in the numbers of SCEs/cell in lymphocytes from groups of 9, 7 and 18 workers employed in 2 workshops (a fibre reinforced plastics boat factory and a polyester-resin board factory) exposed to 2 - 211 ppm styrene in the boat factory and to 30 ppm in the resin board factory. About 30 metaphases were scored for SCE for each individual. Mean numbers of SCEs/cell were 6.7 - 9.1 in these studies.

A group of 18 workers from a glass fibre reinforced polyester plant in Norway was exposed to styrene at up to 44 ppm with a mean of 13 ppm (no further details of exposure given), and cells from whole blood cultures per individual were compared with samples from 9 controls (Hansteen *et al*, 1984). The controls were of the same sex, of similar age and had similar smoking habits. SCEs were scored in 30 cells per person in 70-hour cultures. There was no increase in the number of SCEs/cell relative to the unexposed group (6.0 - 6.9 SCEs/cell found in this study).

In another study, SCE frequency was determined in peripheral lymphocytes from 20 styrene-exposed workers and 20 non-exposed workers employed in 2 different boat-building factories (Kelsey *et al*., 1990). Styrene exposure was assessed by personal monitoring, exhaled breath sampling and urine analysis of mandelic acid levels. Mean airborne styrene concentrations were 48 and 53 ppm for smokers and non-smokers, respectively. Smokers and non-smokers were analysed seperately. No increase in the number of SCEs/cell was found in the exposed workers (6.69) compared to controls (6.62).

Van Hummelen *et al* (1994) determined SCE frequency in samples from 43 exposed workers in a plant manufacturing fibre glass-reinforced plastic pipes and 15 age-matched non-exposed controls from another factory making wooden pallets. Styrene exposure was 7 ppm (reference period not given), range 0.5 - 26 ppm. Lymphocytes were cultured for 72 hours in duplicate and mostly 50 metaphases per person scored. There were no differences in SCE frequency between the two groups. Smoking habits correlated positively with SCE frequency. The numbers of SCEs/cell in this study were 4.4 - 5.6.

Brenner *et al*, 1991 scored the number of SCEs/cell in samples from a group of 10 styrene-exposed GRP workers and 9 unexposed controls. There were significant differences in gender, education, smoking habits and medication between the groups. A styrene concentration of 11 ppm (geometric mean, 8 hour TWA, range 1 - 44 ppm) was measured using personal sampling of the breathing zone. Mandelic acid levels in end-of-shift urine

from all individual were measured to confirm exposure. Fifty metaphases from whole blood cultures were scored per individual from 72-hour duplicate cultures. There were no significant differences in the number of SCEs/cell between exposed and control groups (mean number/cell 9.4 - 10 in this study). This study is deficient because there were significant differences between potential confounding factors in the exposed and control groups.

An increase in SCEs has been reported in peripheral blood lymphocytes of a high-exposure subgroup of 23 male workers exposed to 20 - 230 ppm styrene in a fibre-reinforced plastic boat building factory (personal air sampling, over 3 to 8 hours) (Artuso *et al*, 1995). SCEs were not increased in a low-exposure subgroup of 23 workers exposed to 0.46 - 28 ppm styrene. Control subjects (51) from the same geographical area and with no history of occupational exposure to genotoxic agents were matched by age, sex and smoking habits. Other potential confounding factors such as X-ray exposure, working history, medical history, medical intake, use of alcohol, drugs and smoking status were evaluated. The percentage of smokers in the high exposure group was greater than that in the other groups (60% compared with 45% in controls and 30% in the low exposure group). An average of 75 metaphases per subject were scored. The sample analysis was divided between 2 laboratories, hence a range of results was presented. The mean numbers of SCEs/cell were 2.38 (± 0.12) and 6.57 (± 0.5) in the low exposure group and 3.01 (± 0.12) and 7.32 (± 0.81) in the high exposure groups, as reported from the two labs respectively. Mean control values were 2.38 and 5.44 SCEs/cell for lab 1 and 2 respectively. The marginal increases in SCEs seen in this study in the high exposure group compared to controls are of a magnitude not likely to be biologically significant. Also, the lack of reproducibility between labs casts doubt on the validity of the claimed positive response. Furthermore, the much higher percentage of smokers in the high exposure group confound the interpretation of the results. Overall, no conclusions can be drawn from this study.

A slight, statistically significant increase in the frequency of SCEs in peripheral blood lymphocytes was found in a study of 44 male styrene-exposed workers and 41 ‘unexposed’ control individuals (Karakaya *et al*, 1997). Workers were exposed to styrene for a mean duration of 9.9 years (± 7.1 years) during the lamination of furniture with polyester resin. Exposure levels ranging between 20 - 300 ppm, were measured in the breathing zone of the workers using a stationary sampler (mean exposures measured during lamination was 152 ppm, and 10 minutes post lamination was 30.3 ppm). Workers and controls were matched for age (average age of both 27 years) and smoking habits (both groups contained 70% smokers). All subjects were questioned with respect to their occupational, medical, family and dietary histories. Peripheral lymphocyte samples were incubated for 72 hours at 37^{o}C and 30 second-division metaphases per individual were analysed for SCEs. The mean frequency of SCEs/cell was 6.60 ± 1.56 in laminators (5.1 in non-smokers and 6.75 in smokers) compared with 5.23 ± 1.23 in controls (3.6 in non-smokers and 5.88 in smokers). There was no correlation between exposure levels or exposure duration and SCE frequency. Overall, although statistically significant, the biological significance of the marginal difference reported, particularly in light of the high variability, is doubtful.

Hallier *et al* (1994) analysed the number of SCEs/cell in lymphocytes from 28 male workers exposed to an average of 40 ppm (laminators) or 10 ppm (formers) TWA (averaging time not indicated) styrene and in 2 male controls. The controls were not matched to the exposed group. Fifty metaphases were scored per person in 66-hour cultures. There was a statistically significant increase in the number of SCEs/cell in laminators but not in formers in both smoking and non-smoking subgroups compared with controls. Overall, in laminators there were 10 (± 1.0) SCEs/cell compared with 6.6 (± 1.0) in controls and 7.6 (± 1.5) SCEs/cell in formers. However, the lack of matching controls makes it difficult to interpret the significance of these results.

SCEs were scored in lymphocytes cultured for 72 hours, obtained from whole blood samples taken from 37 workers from 9 different plants manufacturing reinforced unsaturated polyester resin, exposed to 7 - 95 ppm styrene in air (Camurri *et al*, 1993 and 1984). The scenarios were categorised as, electrical, small parts, helmet, tanks and small cabins. Samples from a control group of 13 unexposed individuals selected on the basis of matching for age, sex and smoking habits were assessed simultaneously. None of the subjects (exposed or unexposed) had had recent viral infections, vaccinations or exposure to a clastogenic agent. The number of cells analysed per subject was 30-50. The mean number of SCEs/cell in exposed workers ranged from 10.9 to 19.6, compared with 10.8 - 12.1 in controls. Statistical significance however, was not attained in all working scenarios including that giving rise to the greatest mean number of SCEs/cell. SCE rates in exposed workers were not significantly different from controls in exposure scenarios where styrene air levels were in the range 7 to 46 ppm. A statistically significant increase was only evident in workers in activities where styrene air levels exceeded 49 ppm and were approaching levels > 58 ppm. It is noted that the numbers of workers (2-7) in each plant at all exposure levels were very low. Also, there was no correlation between exposure levels and SCE frequency. Furthermore, other potentially genotoxic workplace exposures, which are likely to be encountered in unsaturated polyester resin manufacture, were not accounted for. Overall, no conclusions can be drawn from these studies.

Yager *et al* (1989 and 1993) measured the number of SCEs in lymphocytes from 48 workers employed at a reinforced plastic boat manufacturing facility and exposed to a mean 8-hour TWA styrene concentration of 15 ppm (range 0.2 - 56 ppm). Information on drug intake, smoking habit and alcohol consumption was obtained. There were no non-exposed controls in the study. Cultures were incubated for 72 hours and 80 metaphases scored per individual. The SCE values ranged from 4.7 - 9.5 per cell, with a mean of 6.4. A statistically significant increase in the number of SCEs/cell with increasing styrene exposure level was apparent but the majority of the contribution to the regression came from smoking. This important confounding factor and the lack of a control group mean that no conclusions can be drawn about the effects of exposure to styrene.

Andersson *et al* (1980) studied samples from a group of 20 styrene-exposed GRP workers and 21 controls matched for age and sex. Workers were subdivided based on measured 8-hour TWA exposure levels, into low and high exposure groups. Individuals in the low exposure groups (n=14) were exposed to an average ambient air concentration of styrene of approximately 32 ppm, whereas individuals in the high exposure group (n=6) were exposed to styrene at levels of approximately 277 ppm. Lymphocytes were cultured for 72 hours and 25 metaphases scored per person. There was a slight increase in the number of SCEs/cell which was statistically significant (8.4 SCEs/cell in the exposed group and 7.5 SCEs/cell in controls) but no difference was observed between the results from low and high exposure subgroups. Other factors such as smoking habit were included in a multiple regression analysis; there were no significant positive correlations with the numbers of SCEs/cell. There was a high failure rate of cultures and/or staining which casts doubt on the quality of the study, and therefore, no conclusions should be drawn from this study.

Tates *et al* (1994) studied 46 workers using unsaturated polyester resins in the production of containers and exposed to styrene at an 8-hour TWA concentration of 17 ppm (range 0 - 142 ppm). Co-exposure to dichloromethane, an established genotoxin, could not be ruled out. A control group of 22 workers matched for age, sex and smoking habits, employed in a machine building factory and not exposed to any other known genotoxin, was also analysed. Workers were questioned regarding health status, prior exposure to ionising radiation, drug use, smoking and alcohol consumption. Peripheral whole blood samples were collected from all individuals and analysed. The mean number of SCEs/cell was statistically significantly increased in the exposed workers (10.2) compared to controls (5.6). No correlation between duration of exposure and SCE frequency was found. The methodology used in this study is

insufficiently described (e.g numbers of cells scored), but the major confounding factor is co-exposure to dichloromethane, such that the potential effect of styrene cannot be discriminated.

In another study, the frequency of SCEs was analysed in peripheral lymphocytes taken from 10 workers exposed to styrene and 21 healthy controls, not matched for sex or smoking (Biro et al., 2002). No information on exposure was provided in the study report. A marginal increase in the number of SCEs/cell was found in the exposed workers (7.9) compared to controls (6.4). However, given the much higher incidence of smokers in the exposed group than in controls, no conclusions can be drawn from this poorly conducted study.

Laffon *et al* (2002) analysed the frequency of SCEs in peripheral blood samples taken from 14 GRP workers employed for more than 7 years and from 30 controls recruited from university staff. A number of potential confounding factors (age, sex, smoking habits, diet, viral infections, medication) were accounted for in the statistical analysis. However, contribution of organic peroxides (typically encountered in GRP manufacture) to workplace exposure could not be excluded. A marginal increase in the number of SCEs/cell was found in the exposed group (3.51) compared to controls (2.55). An increase was also found in High Frequency Cells (5.93 vs 3.03 in controls). Overall, a positive result was obtained in this study.

Teixeira *et al* (2004) evaluated if individual polymorphisms in xenobiotic metabolising enzymes related to the metabolic fate of styrene (CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1) could modulate the levels of SCE in peripheral blood samples taken from 28 GRP workers (employed for a mean of 12 years) and from 28 controls working mainly in office jobs. A number of potential confounding factors (age, sex, smoking and alcohol habits, diet, medication and X-ray exposure) were determined by the administration of a questionnaire. Personal air sampling was performed in the breathing zone for representative working periods. A mean 8-hr TWA of 27 ppm (range 2 – 91 ppm) styrene was determined. Toluene and acetone were also found to be present in the workplace although at levels less than 1% of the styrene concentration. The urinary metabolites of styrene, namely MA and PGA were also measured in samples collected before the start of the work shift. The mean level of MA + PGA in the exposed population was 401 (range 47 – 1490) mg/g creatinine. A significant correlation between styrene concentration in air and urinary MA + PGA levels was found ($r = 0.72$, $p<0.001$). A statistically significant difference in SCE levels between the exposed workers (7.18/cell) and the control group (6.30/cell) was found. CYP2E1 and GSTP1 were found to modulate the baseline levels of SCE in the control group with wild-type control individuals having a higher level of SCE compared with control individuals carrying at least one non-wild type allele. Individuals of the low exposure group (MA + PGA < 400 mg/g creatinine) with GSTM1 null genotype had significantly higher levels of SCEs when compared with GSTM1 non-null individuals. However, this was not observed in the high exposure group (MA + PGA > 400 mg/g creatinine).

In summary, SCEs are one of the major reported positive endpoints in animal studies and are also induced in *in vitro* assays. In humans, there are 10 clearly negative studies, 2 investigations (Laffon *et al*., 2002 and Teixeira et al., 2004) indicating a marginal effect and 11 which are inconclusive, mainly due to inadequate statistics, technical deficiencies (scoring anomalies), possible exposure to other confounding factors and inadequate cell or individual numbers. Overall, on balance, there is no convincing evidence that styrene increases the frequency of SCEs in humans. Furthermore, the lack of convincing data on the induction of SCEs in humans casts doubt that the positive results claimed for other endpoints are in fact due to styrene since the sensitivity of this endpoint to styrene exposure has been demonstrated in animal and *in vitro* systems.

DNA single-strand breaks

As part of a larger study, the levels of DNA single strand breaks (SSB) were measured using the comet assay in the lymphocytes of 9 lamination workers at various time points over a 3 year period (Vodicka *et al*, 1995 and Vodicka *et al*, 1999). A non-styrene exposed referent group of 7 workers in the same factory and a control group of 8 laboratory workers were also assessed. All subjects were non-smokers. Airborne styrene concentrations were assessed by personal monitors on a number of occasions. At the time of the initial onset of the study mean styrene exposure levels were reported as being around 37 ppm. However, mean values declined over a 4 year period down to 16 ppm. Bood styrene and urinary mandelic acid levels were also analysed. Laminators were found to have significantly higher levels of DNA strand breaks than observed in the factory referents at all time points investigated. At the VI time point (the only quantitative data reported) the tail length measurements for laminators was 10.5 compared with 7.1 for referents, the % DNA in the tail for laminators was 7.2 compared with 5.9 for referents and tail moment for laminators was 1.9 compared with 0.6 in referents. An additional group of 13 laminators (including 4 smokers) and a control group of 13 laboratory workers (including 5 smokers) were included in the study at the VI time point only. Smoking was found not to influence the results generated. No correlation between exposure levels and DNA damage was found. Although no cell viability data were presented, the weak effect reported in the more recent publication (1999) is considered a valid, positive result.

These two previous reports were further analysed by Vodicka *et al* (2002a) in terms of cumulative exposures. Unfortunately, this analysis is lacking in detail which makes it difficult to evaluate. The tail length data were analysed in relation to duration of exposure and also in relation to an arbitrary factor (exposure coefficient) which took account of exposure levels over the period of employment and the total exposure time. Both parameters were claimed to be correlated with tail length, but individual data were not presented. DNA damage levels are known to be a function of age, but it is not clear from the paper (particularly as control control data were not presented) how this was taken into account. Overall, there is no convincing evidence that the DNA damage measured in this study was induced as a function of duration of exposure independent of age. Also, there is no information on whether smoking and other potential genotoxic exposures were accounted for.

In the study of Holz *et al* (1995), as detailed above, no exposure-related increases in SSB in lymphocytes, determined by a modified ‘nick translation assay’, were observed in 25 styrene exposed workers from production plants compared with 25 controls. Styrene exposure levels were measured in the range 0.1 to 0.83 ppm. Smoking was found to have no confounding effect on the results obtained.

DNA SSBs were analysed in mononuclear leukocytes taken from 44 styrene-exposed workers at a hand lamination plant and from 19 unexposed office workers at the same factory matched for age and sex (Somorovska *et al*., 1999). Styrene exposure was measured by workplace monitoring (details not given), blood styrene levels and alveolar sampling immediately after leaving the workplace. The exposed group was then divided in 2 subgroups of low and high exposure (mean 8-hour TWA of 13 and 46 ppm respectively). Current exposure to other chemicals was excluded by chemical analysis, and smoking was accounted for. SSB were assessed by the comet assay. Oxidised pyrimidines and damaged purines were also evaluated by including in the test system the endonuclease III and FPG enzymes. An approximately 2-fold increase in DNA damage, as measured by tail DNA content, was seen in both exposed groups compared to controls. No difference was observed between the high and low exposure groups. The incorporation of the enzymes used to detect oxidative damage did not increase the DNA damage levels in the exposed groups, but did it in the controls. This latter finding does not appear to be internally consistent because, if the enzymes used increased damage levels in controls and styrene

also increased DNA damage above control levels, then the styrene exposed groups should have had an increased damage level in the presence of the enzymes compared to without. This point was not addressed by the authors. However, if these latter results are considered together with the lack of a dose-response relationship, it is possible that this picture was the result of some saturation of the endpoint in these experimental conditions.

DNA SSBs were assessed by the alkaline unwinding, hydroxylapetite technique in lymphocyte samples taken from 14 GRP workers and 8 controls (Brenner *et al*, 1991). A styrene concentration of 11 ppm (geometric mean, 8 hour TWA, range 1 - 44 ppm) was measured using personal sampling of the breathing zone. Mandelic acid levels in end-of-shift urine from all individual were measured to confirm exposure. There were significant differences in gender (workers all male, controls 56% male), education (mean 11.6 years in workers, 15.4 in controls), smoking habits (64% smokers or ex-smokers in workers, 55% ex-smokers only in controls) and medication between the groups. There was a statistically significant increase in SSBs (the amount eluted as single strands compared to the total) in the exposed group compared with controls. However, there was no exposure-response relationship when personal exposure measures or cumulative exposure was taken into account. Also, the number of cigarettes smoked per day had a statistically significant effect on the number of breaks in the exposed group. In view of this and the poor control for potential confounding factors, no conclusions can be drawn from this study.

In another study, the incidence of DNA SSBs was assessed by the unwinding technique in peripheral lymphocytes of 17 GRP workers and 17 controls (Maki-Paakkanen *et al*., 1991). Urinary mandelic acid concentrations were used to calculate air levels in the plant (mean level of 69 ppm, 8-hour TWA). A small increase in SSBs was found in the exposed workers compared to controls.

Walles *et al* (1993) used the alkaline elution technique to measure DNA alkaline-labile SSBs in leukocytes from 17 to 23 styrene-exposed workers at a Swedish plastics factory. No control group was included. Samples were taken before the shift began, at the end of a shift and the next morning pre-shift. There was a range of exposures of 0.04 – 20 ppm styrene with a mean of 7 ppm, 8-hour TWA (data not shown). The mean SSB level increased at the end of the shift compared to the pre-shift values. This increase was statistically significant and appeared to be exposure-dependent. However, there was no increase with years of employment and no correlation between measures of exposure and before-shift or next morning SSB levels. Smoking habit (cigarettes/day) was also positively related to increasing numbers of SSBs. Although an increase in SSBs was seen, this approach without the use of a concurrent control population is seriously flawed as diurnal changes (including smoking as shown in this study) can affect this endpoint. SSBs rose to the highest value in an individual taking paracetamol; results for workers on other medication did not show any particular increase. Overall, given the absence of a control group and the lack of raw data, a full evaluation of these findings cannot be made.

In an earlier study, Walles *et al* (1988) used the DNA unwinding techniques to detect SSBs in lymphocytes from 9 styrene-exposed workers (laminators using unsaturated polyester resin in container manufacture) and 8 unmatched controls (office workers). The reporting of this study was brief. Back correlations of levels of end-of-shift urinary metabolite levels (mandelic acid) indicated styrene exposure levels of ~50 - 100 ppm in the ambient air, but no actual measured exposure data were reported. Multiple regression analysis was used to show that single strand breaks correlated positively with levels of styrene metabolites in urine. However, given the brief reporting, it is difficult to assess the significance of these findings.

Laffon *et al* (2002) analysed by the comet assay the levels of DNA damage in peripheral blood samples taken from 14 GRP workers employed for more than 7 years and from 30

controls recruited from university staff. Based on the urinary mandelic acid levels measured in the exposed individuals, a mean styrene airborne concentration of 19 ppm (8-hour TWA) was calculated. A number of potential confounding factors (age, sex, smoking habits, diet, viral infections, medication) were accounted for in the statistical analysis, although there appears to be an interaction with smoking in the exposed workers. A small but statistically significant increase in tail length was found in the exposed group compared to controls. Overall, a positive result was obtained in this study, although contribution of organic peroxides (typically encountered in GRP manufacture) to workplace exposure could not be excluded.

Shamy *et al* (2002) analysed by the alkaline unwinding technique the levels of DNA SSBs in peripheral blood samples taken from 26 GRP workers and from 26 controls recruited from a food preservation plant. Based on the urinary mandelic acid levels measured in the exposed individuals, a mean styrene airborne concentration of 10 ppm (8-hour TWA) was calculated. Workers exposed to confounding factors were excluded from the analysis. A small but statistically significant increase in SSBs was found in the exposed group compared to controls. Also, a correlation between the levels of DNA damage and urinary metabolites was shown. Overall, a positive result was obtained in this study, although contribution of organic peroxides (typically encountered in GRP manufacture) to workplace exposure could not be excluded.

Sperm DNA integrity was investigated in 46 subjects occupationally exposed to styrene and in 27 healthy unexposed controls (Migliore *et al*., 2002). The exposed individuals had worked for at least 2 years during the last 5 years, and continuously for 6 months, in factories producing reinforced plastics. The reference group included healthy male volunteers of comparable mean age, with no previous history of styrene exposure, living in the same area. All subjects were administered a detailed questionnaire regarding their personal, occupational and medical history and life-style. Preliminary information on recent exposure to X-rays, viral infections or genitourinary tract inflammatory disorders experienced in the last 3 months, or current use of medicinal products were used as criteria to exclude subjects from the study. Styrene exposure was determined by measuring mandelic acid (MA) levels in urine specimens obtained at the end of the work shift, in the same week as the semen sample collection. Urinary MA levels ranged from 5.8 to 1428.7 mg/g creatinine, with a median value of 173.6 mg/g creatinine. DNA integrity was analysed using the modified alkaline comet assay for sperm. There were no differences in the results of the standard semen analysis (sperm concentration and morphology) between exposed subjects and the reference group. However, a statistically significant difference in sperm DNA damage was found between the exposed workers (mean tail moment of 1.5) and the unexposed controls (mean tail moment of 0.8), although no correlation with exposure levels was observed. The mutagenic potential of these DNA lesions remains unclear. It is also noted that no increase in the incidence of aneuploidy and diploidy was found by the same authors in the same study population (see Naccarati *et al*., 2003 under “Other studies”).

Another study investigated different profiles of peripheral blood lymphocyte DNA damage (by the comet assay) in relation to polymorphisms of styrene-metabolising enzyme (Buschini *et al*, 2003). DNA from peripheral blood samples was extracted from 48 styrene-exposed workers (exposed to an 8-hour TWA of 37 ppm styrene) and from 14 healthy controls and characterised for GSTM1, GSTT1, GSTP1, EPHX 113 and EPHX 139 genotypes. Levels of urinary styrene metabolites were also measured to characterise internal exposure. Comet assays were performed on freshly-collected blood samples. Further comet assays were done by incubating white blood cells from control subjects with increasing concentrations of styrene oxide or a positive control (ethyl methanesulphonate). There was no statistically-significant differences in the median tail moment of controls and styrene-exposed workers. However, there was a clear difference in distribution of DNA damage (assessed by the 99th and 95th percentiles of reference distributions – no more details given on the source of these

reference distributions). The influence of different styrene-metabolising enzyme genotypes on the levels of DNA damage was only assessed in the styrene-exposed workers as there were insufficient numbers of controls for meaningful analysis. Correlations between the levels of urinary styrene metabolites and the levels of DNA damage were slightly affected by the individual's genotypes. For example, GSTM1 positive styrene workers produced slightly different amounts of mandelic acid and phenyl glyoxylic acid and had slightly different levels of DNA damage compared to GSTM1 null styrene workers. However, there was considerable inter-individual variation and the correlations were not strong (eg. r^2 values were less than 0.4. Overall, in this study, occupational exposure to styrene was associated with increased DNA damage. Also, a slight effect of styrene-metabolising enzyme genotypes on levels of urinary styrene metabolites and on DNA damage levels was observed.

In summary, a number of different assay types have been used to detect DNA strand breaks, including the DNA unwinding assay and the comet assay. The former was used by Maki-Paakkanen *et al* (1991), Brenner *et al* (1991) and Shamy *et al* (2002). A small increase in SSBs was reported in all studies. No statistically significant correlation was seen between the levels of damage and urinary metabolites or blood styrene levels in the first two studies, although a correlation was claimed in the third. Walles *et al* (1993) used the more sensitive alkaline elution assay to compare increases in SSBs at the end of shifts relative to pre-shift values. Although an increase was seen, this approach without the use of a concurrent control population is seriously flawed as diurnal changes (including smoking as demonstrated in this study) can affect this endpoint. Therefore, the increase in damage cannot be ascribed to styrene without an adequate control group. Other studies have used the comet assay to investigate DNA damage (Vodicka *et al*., 1995, 1999, 2002a, Somorovska *et al*., 1999, Laffon *et al*., 2002, Migliore *et al*., 2002, Buschini *et al*., 2003). It is not clear how independent the first 4 publications are since the paper of Vodicka *et al* (2002a) appears to be an overview of all of the other papers and there is clear overlap between study groups and data. Four of the studies appear to indicate that styrene induces DNA damage (Somorovska *et al*., 1999, Vodicka *et al*., 1999, Migliore *et al*., 2002, Buschini *et al*., 2003) although there are some deficiencies in two of them (internal data inconsistency in Somorovska *et al*., 1999 and lack of viability data in Vodicka *et al*., 1999). The positive response reported by Laffon *et al* (2002) appears to be due predominantly to smoking. There is no evidence of a relationship to exposure levels in any study. These assays are considered to be indicators of damage and various parameters which are not considered to be genotoxic such as exercise, diurnal sampling period and vitamin C intake have been previously shown to induce responses (e.g. Moller *et al*., 2000). Thus, although there is some evidence for DNA damage in styrene exposed workers, the studies have not been consistent, the levels of damage are not related to exposure levels and the significance of any positive response is unclear.

Other studies

No significant effects on uninduced UDS *in vivo* were reported in a study of the lymphocytes from 38 male workers (mean age 39 years) occupationally exposed to styrene air concentrations of 1 - 40 ppm for 1 to 23 years, during the manufacture of fibreglass reinforced polyester plastics (Pero *et al,* 1982). Air samples were collected via personal, static and monitoring sampling procedures at the time of blood sampling. Samples from a control population of 20 individuals (mean age 36 years), from the same town, were tested simultaneously. There were 17 smokers in the exposed group and 8 in the control group. UDS in lymphocytes was measured by ^{3}H-thymidine incorporation following hydroxyurea treatment to stop replicative DNA synthesis and induction by two well-known genotoxic agents, N-acetoxy-2-acetylaminofluorene (NA-AAF) and UV. UDS induced by NA-AAF was greater in the lymphocytes from styrene-exposed workers compared to controls; no increase was seen with UV-induced UDS. The interpretation of this finding is difficult as the study

looked at the modulation of DNA repair activity induced by two other genotoxic agents in lymphocytes of styrene-exposed workers and unexposed controls and it did not address DNA damage caused directly by styrene. Also, the UDS methodology employed in the study has been discredited as not sufficiently specific since replicative DNA synthesis can occur despite the hydroxyurea block. Overall, no conclusions can be drawn from this study.

The frequency of aneuploidy and diploidy was investigated in spermatozoa of 18 subjects occupationally exposed to styrene and in 13 healthy unexposed controls (Naccarati *et al*., 2003). The exposed individuals had worked for at least 2 years during the last 5 years, and continuously for 6 months, in factories producing reinforced plastics. The reference group included healthy male volunteers of comparable mean age, with no previous history of styrene exposure, living in the same area. All subjects were administered a detailed questionnaire regarding their personal, occupational and medical history and life-style. Preliminary information on recent exposure to X-rays, viral infections or genitourinary tract inflammatory disorders experienced in the last 3 months, or current use of medicinal products were used as criteria to exclude subjects from the study. Semen samples were obtained from 46 styrene-exposed workers and 27 unexposed subjects, but only those (18 exposed workers and 13 unexposed individuals) which had normal parameters (number of sperm $>20*10^6$/ml and volume = 2 ml) were selected. Styrene exposure was determined by measuring mandelic acid (MA) levels in urine specimens obtained at the end of the work shift, in the same week as the semen sample collection. Urinary MA levels ranged from 20.8 to 947.8 mg/g creatinine, with a median value of 292.5 mg/g creatinine. The frequency of aneuploidy and diploidy was analysed by fluorescence in situ hybridisation (FISH) using centromere-specific DNA probes for chromosome X, Y and 2. The incidence of aneuploidy and diploidy for the tested chromosomes did not show a statistically significant difference between workers and controls. However, since the sensitivity of this fairly new approach was confirmed by its ability to detect differences in aneuploidy due to age and smoking, this study provides some evidence that styrene does not alter chromosome distribution in the germ cells of occupationally exposed males. This is consistent with the generally negative response in the human lymphocyte micronucleus biomonitoring studies, where a potential to alter this endpoint is also evaluated.

DNA (and protein) adducts

In a 3-year study of lamination workers the persistence of styrene DNA adducts in lymphocytes and granulocytes has been investigated, using the ^{32}P post-labelling method (Vodicka *et al*, 1994; Vodicka *et al*, 1995; Vodicka *et al*, 1999). Further study details are reported in the section (above) reviewing the chromosome aberration studies conducted by the same authors on the same population.

In granulocytes no significant differences in styrene specific O^6-guanine adduct levels between laminators and control groups were observed at any time point. In lymphocytes styrene specific O^6-guanine adduct levels were significantly higher in laminators (5.9 adducts/10^8 nucleotides) than in factory controls (1.4 adducts/10^8 nucleotides) and in laboratory controls in whom levels were negligible (below the limits of detection). There were no significant time-related differences in magnitude of the adduct levels measured in laminators or in controls at any of the time points investigated (sample time I – 5.9/10^8 nucleotides and 1.4/10^8 nucleotides, II – 4.9/10^8 nucleotides and 0.7/10^8 nucleotides, III – 5.4/10^8 nucleotides and 1.0/10^8 nucleotides, IV – 6.0/10^8 nucleotides and 0.8/10^8 nucleotides and 0.9/10^8 nucleotides, in laminators and factory controls respectively).

As part of the study an additional group of 13 laminators (including 4 smokers) and a control group of 13 laboratory workers (including 5 smokers) were included in the study at the VI time point only. These samples were analysed in addition for the presence of N-terminal valine adducts. The levels of both adducts were increased when compared with controls (O^6-

styrene adduct levels were 5.9/10^8 dNp in laminators compared with 0.7/10^8 dNp in controls, and N-terminal valine adducts levels were 1.7 in laminators compared with 0 in controls). Smoking was found not to influence the results generated for either adduct type (O^6-styrene adduct levels were 2.5/10^8 dNp in smoking laminators compared with 0.2/10^8 dNp in smoking controls, and N-terminal valine adducts levels were 1.9 in smoking laminators compared with 0 in smoking controls).

These three previous reports were further analysed by Vodicka *et al* (2002a) in terms of cumulative exposures. Unfortunately, this analysis is lacking in detail, which makes it difficult to evaluate. The DNA adduct data were analysed in relation to duration of exposure and also in relation to an arbitrary factor (exposure coefficient) which took account of exposure levels over the period of employment. Both parameters were claimed to be correlated with DNA adduct levels, but individual data were not presented. DNA adduct levels are known to be a function of age, but it is not clear from the paper (particularly as control data were not presented) how this was taken into account. Also, there is no information on whether smoking and other potential genotoxic exposures were accounted for. Overall, there is no convincing evidence that the DNA adducts measured in this study were induced as a function of duration of exposure independent of age.

In a methodological investigation, Otteneder *et al* (1999) systematically optimised the post-labelling procedure coming to the conclusion that adduct levels in human lymphocytes on the order of five adducts per 10^8 nucleotides should be viewed with caution.

In the study by Holz *et al* (1995), as detailed above, no exposure-related increases in total or segregated monocyte DNA adducts were observed in 25 styrene exposed workers in styrene production plants compared with 25 controls, measured using the ^{32}P-postlabelling technique. Four different adducts were detected, but not identified. Styrene exposure levels were < 1 ppm. There was considerable variability between individuals within both test groups. Smoking was found to have no confounding effect on the results obtained, but confounding exposures to other aromatic hydrocarbons (ethylbenzene, benzene, toluene, xylines) were not accounted for.

White blood cells samples were taken from a group of 47 GRP workers (boat building factory) and ^{32}P-postlabelling technique used to detect DNA adducts (Horvath *et al*, 1994). There were no controls but some workers were exposed to relatively low mean styrene concentrations; overall mean 8-hour TWA exposure was 16 ppm, range 1 - 56 ppm. Standards were made by reacting DNA with styrene oxide *in vitro*; three styrene oxide-DNA adducts were identified. One of the two adducts found in samples from workers was identified by co-chromatography as N-(2-hydroxy-1-phenylethyl)-2-dGMP. However, definitive identification of the adducts by GC-MS was not reported. The mean levels of this adduct were 15.8/10^8 nucleotides; levels of the second unidentified adduct were 14.2/10^8 nucleotides. There was a very wide range of adduct levels in the samples, and no correlation between exposure levels and adduct levels was found.

Workers at two GRP plants were included in another ^{32}P-postlabelling study (Vodicka and Hemminki, 1993). A standard was synthesised by reacting styrene oxide with dGMP and forming an O^6-dGMP product. The number of exposed workers was 10 at one of these GRP plants with 8 controls. The controls were of similar age and smoking habit to the exposed workers. Thirteen exposed workers and 10 controls (no further details given) were selected from the other plant. Average styrene exposure levels were 88 ppm and 50 ppm (reference period not given) for plant 1 and 2 respectively. Chromatography was used to assess the identity of the adducts in DNA from white blood cells, and co-elution with the standard O^6-dGMP adduct was observed. There were 4.7 O^6-dGMP adducts/10^8 nucleotides in the first exposed group and 7.3 O^6-dGMP adducts/10^8 nucleotides in the second. The level of

radioactivity on control chromatograms equated to 0.3 adducts/10^8 nucleotides and 1.1 adducts/10^8 nucleotides.

DNA was isolated from the lymphocytes of one worker exposed to styrene during the manufacture of glass reinforced plastics and DNA adducts detected by the ^{32}P-postlabelling method (Liu *et al*, 1988). On the day of testing the average air concentration of styrene as an 8 hour TWA, for the exposed individual was measured at 96 ppm. Five styrene oxide-DNA adducts were characterised *in vitro* in the same laboratory; two such adducts were found in the sample from the worker.

DNA isolated from the lymphocytes of a worker (exact occupation not specified) exposed to 47 ppm styrene over an 8 hour period was analysed for the presence of styrene-oxide DNA adducts by the ^{32}P-postlabelling method (Bodell *et al*, 1990). No control worker samples were analysed. Several adducts which appeared to be similar to previously identified styrene-oxide adducts, were detected in the worker sample. However, no assessment for smoking was conducted. Therefore it is unclear if the adducts identified resulted from styrene exposure or from smoking. No conclusions can be drawn from this limited study.

A study of boat builders by Marczynski *et al* (1997) has indicated that in 17 exposed workers the mean level of 8-hydroxy-2'-deoxyguanosine adducts (8-OhdG) (2.23/10^5 dG) in white blood cells was statistically significantly increased compared to that determined in 67 control individuals (1.52/10^5 dG). Controls were healthy individuals and were matched for age. No quantitative assessment of styrene exposure levels was reported. No significant differences between the incidences of 8-OhdG adducts in smokers and non-smokers, of either the workers or the control population were found. In workers, 11/17 had been exposed to styrene for > 10 years. These workers had a level of 8-OhdG adducts (2.31/10^5 dG ± 6.2) which was higher than in the other 6 workers exposed for < 10 years (2.11/10^5 dG ± 0.36). However, it is noted that the longer exposed group was also older and older controls also showed an elevated frequency. The type of adduct assessed in this study is produced by oxidative damage to DNA. Thus, although there is some evidence of a weak increase of this oxidative adduct in the exposed workers, this may be completely unrelated to styrene exposure. Hence, no conclusions can be drawn from this study.

In another study, 1-adenine styrene DNA adducts were investigated by ^{32}P-postlabelling with hplc in lymphocytes taken from 9 styrene exposed workers at a hand-lamination plant and 11 controls from a research institute matched for age, sex and smoking habits (Koskinen *et al*., 2001). Styrene exposure was assessed by personal dosimeters, alveolar air samples and urine measurements. Mean styrene exposure level was 18 ppm (8h TWA). Three exposed workers were found to have detectable levels of these adducts (mean level of 0.79/10^9 nucleotides). In the other 6 exposed workers and in controls, these adducts were below the limit of detection. No correlation between exposure and adduct levels was found. The authors also compared the results of this investigation with the levels of adducts found in workers exposed to butadiene. The adduct levels found in the styrene exposed workers were significantly lower than those found in the butadiene exposed workers.

No exposure-related increases in styrene-7,8-oxide haemoglobin adducts were found in a field study of 52 exposed GRP workers and 24 unexposed control individuals (Severi *et al*, 1994). Individuals were questioned regarding smoking habits, alcohol and caffeine consumption and medical history (including any X-ray treatments). The styrene exposures and end-of-shift urinary mandelic acid levels of each individual were assessed once a week over a 4 week period. At the end of the study period blood samples were taken from each individual and analysed for styrene oxide adduct formation on the N-terminal valine in haemoglobin by gas chromatography and mass-spectrometry (limit of detection 10 pmol/g). Personal air samples of the GRP workers showed an average styrene exposure of 7 ppm

(range 0.5 to 25 ppm). No details of the reference period of sampling were reported. Urinary mandelic acid levels confirmed styrene exposure in the workers.

Styrene oxide adducts have been measured in the blood haemoglobin and blood albumin of 48 healthy workers (male and female) employed in boat manufacturing (Yeowell-O'Connell *et al*, 1996). Subjects were recruited regardless of expected intensity of exposure or smoking status. The only pre-requisite for inclusion was that they had been employed in the current job for at least 1 year. Personal exposure levels over a shift were measured 7 times over a 1 year period. Styrene exposure levels ranged from 0.2 - 54 ppm (average 15 ppm). Blood samples were collected on the day of exposure measurement, haemoglobin and albumin fractions were analysed for the presence of styrene oxide adducts. No evidence of any exposure-related increase in haemoglobin adducts was found. However, an increase in the presence of albumin adducts with exposure was observed. Given that the workers may also have been exposed to styrene oxide, at exposure levels of up to 525 µg/m3 over a shift, and that smoking was not addressed as a cofounder, the validity of these findings as being indicative of styrene exposure is uncertain.

Styrene oxide adducts (albumin and haemoglobin) have been measured in the blood and urine of 22 male workers (potentially exposed to high levels of styrene) in the fibreglass reinforced plastics industry (3 factories) and in 15 unexposed controls from hospital donors or staff (Fustinoni *et al*, 1998). The mean age of the workers was 30 years compared to 33 years for controls. Smoking status was determined by questionnaire; 14 workers and 3 controls were smokers. No further information was given on matching between the groups. Subjects were recruited regardless of expected intensity of exposure or smoking. The only pre-requisite for inclusion was that they had been employed in their current job for at least 1 year. Blood and urine samples were taken 15 hours post last exposure (just prior to the next shift). An airborne styrene exposure level of 23 ppm was predicted from the levels of metabolites (mandelic and phenygloyoxylic acid) measured in the urine. Haemoglobin and albumin fractions were analysed for the presence of styrene oxide adducts. No evidence of any exposure-related increase in styrene oxide protein adducts was found. Mean levels of adducts were 3.44 nmol/g albumin and 5.84 nmol/g Hb in workers compared with 3.24 nmol/g albumin and 5.66 nmol/g Hb in controls.

In summary, there are several studies which have shown the presence of styrene-related DNA adducts in humans. The majority of these have been conducted in lymphocytes or mononuclear cells. Studies using granulocytes or monocytes have been very weakly positive or negative, presumably a reflection of the quick turnover of these cell types which makes them unable to indicate cumulative exposure. Some of the studies have failed to identify the adducts detected. However, to date, adducts at N2-guanine, O6-deoxyguanosine, 1-adenine have been identified. The major adduct seen in animal studies, N7-styrene oxide-guanine, has not been detected so far in human studies, presumably partly due to its short half-life. The adducts seen, which may be minor but stable adducts, are present at low levels and some comparisons have been made of their significantly lower levels in relation to those found in butadiene exposed workers (Koskinen *et al*., 2001). Marczynski *et al* (1997) studied oxidative adducts in styrene exposed workers and proposed this as the mechanism of styrene genotoxicity. However, the possibility of confounding environmental exposures to oxidative mutagens has not been adequately characterised in their work. Overall, there is good evidence that styrene induces DNA adducts in humans but the significance of these in terms of subsequent mutagenicity is not proven.

Summary of human studies

A large number of studies have been published which have aimed to investigate the genotoxic potential of styrene in humans by examination of various endpoints in styrene

exposed workers. Very low levels of DNA adducts were found in some styrene exposed workers but it has been stated that such low levels should be viewed with caution. There is also some evidence of DNA damage (SSBs) induced in styrene exposed workers. Both these endpoints are indicative of exposure but are not necessarily associated with heritable effects. The results of several studies on another indicator endpoint of unclear health significance, SCEs, did not provide evidence of a positive response, despite these being induced in animals exposed to styrene. There are also many studies investigating endpoints (gene mutations, chromosome aberrations and micronuclei) known to lead to heritable effects. The number of studies assessing gene mutation is very limited and no conclusions can be drawn from them. Although 5 studies appear to present evidence that styrene may be weakly clastogenic in humans (Brenner *et al*., 1991; Tomanin *et al*., 1992; Anwar and Shamy, 1995; Somorovska *et al*., 1999; Laffon *et al*., 2002), there are 11 robust negative studies also. Together with a lack of evidence of a dose-response relationship and the negative response for induction of micronuclei when studied concurrently in two of the positive chromosome aberration studies, no clear conclusion on *in vivo* clastogenicity of styrene in humans can be made.

Overall, given the lack of evidence of consistent relationships between exposure levels and study outcome, the lack of any consistent profile of endpoints and the absence of information on the relevance of the types of adducts seen and their mutagenic potential *in vivo*, there is no convincing evidence that styrene has shown mutagenic activity in humans.

4.1.2.7.4 Summary of genotoxicity

A large number of studies have been published which have aimed to investigate the genotoxic potential of styrene in humans by examination of various endpoints in styrene exposed workers. Very low levels of DNA adducts were found in some styrene exposed workers but it has been stated that such low levels should be viewed with caution. There is also some evidence of DNA damage (SSBs) induced in styrene exposed workers. Both these endpoints are indicative of exposure but are not necessarily associated with heritable effects. The results of several studies on another indicator endpoint of unclear health significance, SCEs, did not provide evidence of a positive response, despite these being induced in animals exposed to styrene. There are also many studies investigating endpoints (gene mutations, chromosome aberrations and micronuclei) known to lead to heritable effects. The number of studies assessing gene mutation is very limited and no conclusions can be drawn from them. Although 5 studies appear to present evidence that styrene may be weakly clastogenic in humans, there are 11 robust negative studies also. Together with a lack of evidence of a dose-response relationship and the negative response for induction of micronuclei when studied concurrently in two of the positive chromosome aberration studies, no clear conclusion on *in vivo* clastogenicity of styrene in humans can be made.

Overall, given the lack of evidence of consistent relationships between exposure levels and study outcome, the lack of any consistent profile of endpoints and the absence of information on the relevance of the types of adducts seen and their mutagenic potential *in vivo*, there is no convincing evidence that styrene has shown mutagenic activity in humans. Hence, information from studies in experimental animals and other systems needs to be considered.

The overall picture presented by the *in vitro* assay results available is that at least in some text systems (including Ames tests *in vitro* chromosome aberration studies in mammalian cells), styrene does posses some genotoxic potential *in vitro*. Metabolic activation (presumably to styrene oxide) is required for this activity. Styrene has been exhaustively studied in clastogenicity studies in animals up to dose levels producing severe toxicity in some cases. There is no convincing evidence of styrene clastogenicity when the quality of the studies and the plausibility of the test results are considered. Equivocal results were obtained after exposure to high doses causing lethality. However, overall, negative results

were obtained from *in vivo* chromosome aberration and micronucleus studies in the rat, hamster and the mouse following single or repeated exposures to styrene up to concentrations and/or doses causing systemic toxicity, via the inhalation, oral and intraperitoneal route in the tissues examined (bone marrow, peripheral lymphocytes, splenocytes and whole blood). Furthermore, a recently published micronucleus test in bone marrow cells of mice conforming to the current OECD guideline was clearly negative.

The general pattern of SCE results in the wide range of tissues examined (lymphocytes, spleenocytes, bone marrow, alveolar macrophages, regenerating liver cells) from both the rat and the mouse following inhalation or i.p exposure to styrene has been positive. However, it is important to note that, in most cases, concomitant chromosome aberration and/or micronucleus assays involving the same animals and in some cases the same tissues were carried out and that negative results were obtained for these indicators of chromosome damage. Therefore, this clearly reduces the significance of the SCE findings in relation to mutagenicity.

The binding of styrene metabolites to DNA was very low and did not indicate any specificity for the target tissue (mouse lung). Induction of alkali-labile single-strand breaks has also been produced *in vivo* in rats and mice exposed to styrene. Again the significance of these findings is unclear, given the repeated failure of styrene to demonstrate mutagenic activity in standard clastogenicity assays.

In summary, the available data suggest that styrene is weakly positive in indicator tests detecting SCEs, DNA stand breaks and DNA adducts. In contrast, an in vivo UDS test performed in accordance with international guidelines did not reveal a genotoxic effect of styrene in mouse liver.

Overall, based on standard regulatory tests, there is no convincing evidence that styrene possesses significant mutagenic/clastogenic potential *in vivo* from the available data in experimental animals.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

Inhalation

Rat

In a well conducted combined chronic toxicity/carcinogenicity study, groups of 70 male and 70 female Sprague Dawley rats were exposed whole-body to 0, 50, 200, 500 or 1000 ppm styrene monomer vapour (97.7 to 99.5% purity) for 6 hours/day, 5 days/week for 104 weeks (Hardy *et al*, 1996 and Cruzan *et al*, 1998). Ten animals per sex per group were sacrificed at week 52 for interim analysis. The remaining 60 animals/sex/group were maintained for the remainder of the study duration.

The animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. Blood samples were taken from 5 animals per sex per group for extensive haematological and biochemical analysis at 8-week intervals. Urine samples were taken from the remaining animals for extensive analysis at weeks 13, 26, 52, 78 and 104. Prior to termination animals underwent ophthalmoscopic examinations. At necropsy, all standard organs were weighed, macroscopic, observations on a wide range of tissue and organs (including lung and nasal tissue) recorded and extensive histopathological examination performed.

At 104 weeks the number of decedents per group (0, 50, 200, 500 or 1000 ppm) were; 25, 24, 32, 24 and 29 in males and 30, 32, 31, 19 and 11 in females, respectively, with no evidence that styrene exposure increased mortality. The only clinical sign of toxicity observed was increased salivation with restlessness followed by hunched posture, in animals exposed to 500 and 1000 ppm, from week 19 and 1, respectively, for the initial 10 – 30 minutes post-exposure. Details of systemic effects and non-neoplastic findings are presented in section 4.1.2.6.1.

In rats examined after 104 weeks which were exposed to 500 and 1000 ppm, relative to controls there was a higher incidence of benign interstital cell tumours in the testes (in 14% and 19% of animals respectively compared to 6% in controls). Incidences in other exposure groups were 6, 5 and 4% at 0, 50 and 200 ppm, respectively. It is reported that comparison with the historical background range for this species in this laboratory indicated that the incidences in all groups were within the normal range (historical incidence data were not presented). In view of this and in the absence of evidence for styrene producing other changes (hyperplasia, atrophy) associated with benign interstitial cell tumours, these testicular tumour observations are considered to be chance findings and not treatment-related. No increases in the incidence of mammary tumours in females were observed in any animals at exposure concentrations up to and including 1000 ppm. This observation is significant in relation to the studies below. There were no other significant findings in relation to tumour incidence. Overall, these data indicate that styrene was not tumourigenic in the rat following lifetime inhalation exposure to concentrations of up to1000 ppm.

In another inhalation study in rats, groups of 30 male and 30 female Sprague Dawley rats were exposed to 25, 50, 100, 200 or 300 ppm styrene, for 4 hours/day, 5 days/week for 12 months (Conti *et al* 1988). Control groups of 60 animals per sex received air only. This study was reported only briefly, without mortality data. Animals were kept for their life-span before full necropsy. Signs of toxicity were explored fortnightly and bodyweight measured every 2 weeks (during exposure for 12 months) or 8 weeks (subsequently). Survival was claimed to be not significantly different between groups. No differences in bodyweight gain occurred. At 100 ppm, but not at 200 or 300 ppm, there was a statistically significantly higher incidence of total malignant tumours, but individual organ or tissue-specific tumours were not statistically significantly increased at this dose level, suggesting that a genuine effect of styrene was not present. In females, a higher incidence of total (including benign) mammary tumours and malignant mammary tumours was reported in all treated groups. It was claimed by the authors that the increased incidence of such tumours was treated-related and statistically significant. However, the results do not show a clear exposure-response and a statistical analysis was not presented. The frequency of malignant mammary tumours was reported as percentages: 10, 20, 13, 30, 40 and 30% in the 0, 25, 50, 100, 200 and 300 ppm groups respectively. For total mammary tumours the incidences were 57, 80, 70, 77, 80 and 83%. Unfortunately, historical control data were not given. No meaningful conclusions can be drawn from this erratic pattern in the incidence of commonly occurring background tumours. Overall, there is a lack detail in the reporting of this study and it does not meet current regulatory standards in terms of the numbers of animals and the exposure regime used. Therefore in terms of evaluating the carcinogenic potential of styrene in rats, this study is considered to have shown no evidence of styrene carcinogenicity, but to have been of limited value.

A chronic inhalation study has been carried out in groups of 85 male and 85 female Sprague Dawley rats (Jersey *et al*, 1978). Two exposure levels were investigated. The high exposure group was exposed to 1200 ppm for the first two months but this produced marked signs of toxicity (narcosis, anaesthesia and death in three animals), and was reduced to 1000 ppm for the rest of the exposure period. The low exposure group was exposed to 600 ppm and there was also a negative control group. All exposures were for 6 hours/day, 5 days/week.

The males were exposed for about 18 months and the females for 21 months. The study was terminated at 24 months.

There was a reduction in the rate of bodyweight gain at both styrene exposure levels, but no increased mortality was noted (apart from the three rats dying at 1200 ppm). An outbreak of chronic pneumonia particularly affected the control animals and resulted in this group having a higher mortality. Only 29 of the control males survived to 18 months as compared to 47 and 66 of the males from the high and low exposure groups respectively. The corresponding figures for the females were 48 of the controls as opposed to 40 and 53 of the styrene-exposed animals. This low survival in male controls due to an outbreak of chronic pneumonia compromises the valididty of the entire study as it indicates that control animals and those exposed to styrene were either kept in different conditions or were heterogeneous in other aspects. Mammary adenocarcinomas were higher than in the controls in females in the 600 ppm group only, but the results were still within the historical control range. In both treated groups, the combined incidence of leukaemia and lymphosarcoma in females was higher than in controls but the difference was not statistically significant. A high incidence of autolysis was noted in the autopsy samples, with about 30% being rated as having between “slight to moderate” or “severe” autolysis. However, it was stated that reliable identification of neoplastic processes was possible in most cases and no animals were therefore considered lost from the study. In view of the marked reduction in survival of males due to chronic pneumonia infection, interpretation of the results of this study is compromised, and no conclusions should be drawn.

Mouse

A well conducted combined chronic toxicity/carcinogenicity study is available, in which groups of 70 male and 70 female CD1 mice were exposed whole body to 0, 20, 40, 80 or 160 ppm styrene monomer vapour (98.8 to 99.5% purity) for 6 hours/day, 5 days/week for up to 104 weeks (Coombs *et al*, 1998, Cruzan *et al*, 2001). Ten animals per sex per group were sacrificed at weeks 52 and 78 for interim analyses. The remaining 50 animals/sex/group were maintained for the remainder of the study duration.

The animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. Blood samples were taken from 5 animals per sex per group for extensive haematological and biochemical analysis at 8-week intervals. Urine samples were taken from the remaining animals for extensive analysis at weeks 53, 79 and 97 (females) or 104 (males). Prior to termination animals underwent ophthalmoscopic examination. At necropsy, all standard organs were weighed, macroscopic observations on a wide range of tissue and organs recorded and extensive histopathological examination performed.

Two females at 160 ppm were found dead or moribund during week 2 of exposure. Deaths were considered to be treatment-related and as a consequence of the hepatocyte necrosis observed at necropsy. Discoloured liver was also noted in 1 female. No other treatment-related deaths were observed. Due to the increased mortality in control females, terminal sacrifice of the females was brought forward to week 97, whereas for males it was maintained at week 104. At weeks 104 and 97 the number of decedents per group were; 14, 23, 23, 14 and 17 males and 23, 18, 17, 16 and 15 females, respectively. The only clinical sign of toxicity observed was increased activity in animals at 160 ppm, for the first week of exposure, compared to controls. Activity levels were comparable with controls throughout the remainder of the study. Details of systemic effects and non-neoplastic findings are presented in section 4.1.2.6.1; of particular note was the observation in the lung of early Clara cell toxicity followed by cell proliferation and sustained hyperplasia.

To examine the progression of any chronic toxicological effects, 10 predesignated mice of each gender from each treatment group were killed after 52 and 78 weeks of exposure. There was no evidence of any treatment-related neoplastic findings at the week 52 interim

sacrifice. At the week 78 interim sacrifice, an increase of pulmonary bronchiolar-alveolar adenomas was seen in some of the styrene exposed mice. Such increases however failed to reach statistical significance with no evidence of a dose-related effect.

At week 97/104 (terminal sacrifice) a statistically significant, but not smoothly dose-related increase in the incidence of pulmonary bronchiolar-alveolar adenomas was observed in males at 40 ppm and above (with incidences of 30, 42, 70, 60 and 70% at 0, 20, 40, 80 and 160 ppm, respectively) and in females at 20 ppm and above (with incidences of 12, 32, 32, 22 and 48% at 0, 20, 40, 80 and 160 ppm, respectively). The incidence of bronchiolar-alveolar carcinomas was significantly increased (as compared to controls) in female mice exposed to 160 ppm with 14% of treated animals being affected. Numbers of animals with adenocarcinomas failed to reach statistical significance in the other female treatment groups and in the male mice exposed to styrene. The historical control range for this tumour type in female and male mice of this strain is 0-13% and 7-26% respectively. There were no significant differences between styrene-exposed and control animals for tumours at other sites.

The results of this investigation showed that female and male CD-1 mice exposed via inhalation to $\geq$ 20 ppm styrene for 98 and 104 weeks respectively exhibited a significant increase in bronchiolo-alveolar adenomas. A statistically significant increase in bronchiolo-alveolar carcinomas was also seen in female mice only at the highest concentration (160 ppm). In relation to possible sex differences, data from recent repeat dose studies specifically designed to investigate the effects of styrene in the mouse lung (see section 4.1.2.6) have also demonstrated that female CD-1 mice are more susceptible than males to styrene-induced lung damage (Green, 1999a). The observed increases in lung tumour incidence occurred in conjunction with early Clara cell toxicity followed by cell proliferation and sustained hyperplasia (see section 4.1.2.6).

Subsequent to the findings of the above study, immunohistochemical analyses of the primary lung tumours were undertaken (Brown *et al*, 1999). Sections from the lung tumours in control and high dose animals were stained for Clara cell (CC16) and Type 2 surfactant cell markers. The location of the tumour relative to the bronchioles was also determined.

Immunohistochemical analyses showed that 100% of the tumours in control and exposed animals were to some degree positive for surfactant, whereas most tumours were negative for Clara cells (<5% positive). There was no difference in the staining reactivity for either cell marker between control and exposed tissue samples. The evaluation of the location of tumours showed no clear relationship as to whether the tumours occurred in the prebronchiolar region or had a bronchiole with the tumour. These findings show that the bronchiolo-alveolar tumors observed in the mouse chronic bioassay all had some degree of positive staining for surfactant and not CC16, suggesting that they were predominantly of a Type II cell phenotype rather than Clara cells. Conversely all relevant toxicological effects after exposure periods ranging from 3 days up to 18 months were only observed in Clara cells indicating that these are the target cells of styrene mouse lung toxicity/carcinogenicity. There is evidence in the literature suggesting that this is only an apparent contradiction and that styrene-induced mouse lung tumours are most likely to originate from Clara cells. It has been observed that CC16 expression is lost in the carcinogenic transformation process of Clara cells and that pulmonary adenocarcinomas contain lamellar bodies which are the source of lung surfactant. This evidence provides a very plausible explanation as to why the styrene-induced mouse lung tumours originating from Clara cell toxicity do not stain positive for the typical Clara cell CC16 protein but stain positive for surfactant markers.

Oral

Rat

Groups of 50 male and 50 female Fischer 344 rats were treated with 1000 or 2000 mg/kg styrene by gavage for 5 days/week for 78 weeks, and a third group was given 500 mg/kg for 5 days/week for 103 weeks (NCI, 1979). All doses were given as a solution in corn oil and a control group of 40 males and 40 females received vehicle only. The animals were observed until week 104 when the experiment was terminated. A marked reduction in survival was noted at 2000 mg/kg, only 6/50 (12%) of the male rats surviving for 53 weeks and 7/50 (14%) female rats surviving at week 70. No effects on survival were noted at the other dose levels, with 88-94% of the animals surviving for at least 90 weeks. The only compound-related lesion noted at autopsy was hepatic necrosis in animals given 2000 mg/kg. No increase in tumour incidence was noted at any dose level. There was no evidence for any carcinogenic effect in this study.

A briefly reported study in the Sprague-Dawley rat is also available (Conti *et al*, 1988). Some details of this study are the same as those described above in the inhalation study by the same authors. Forty male and forty female animals received 0, 50 or 250 mg/kg in olive oil, 4-5 days/week for 12 months. Apparently there was higher mortality in the highest dose level group. Slightly higher values for the incidences of total mammary tumours in females, malignant mammary tumours in females and leukaemia in males and females combined were reported at 50 mg/kg bw (75, 15 and 3.7%, respectively) but not at 250 mg/kg bw (38, 13 and 2.5%, respectively) compared with concurrent controls (60, 13 and 1.2%, respectively). Given the small differences involved and the inverse dose-response trend, there is no evidence of a styrene-induced effect here. Overall, this is an inadequately reported study; no evidence for any styrene-induced carcinogenicity was apparent.

A 2-year continuous-exposure study has been conducted in the Sprague-Dawley derived rat (Beliles *et al*, 1985). Styrene was administered in drinking water at 0, 125 or 250 ppm. Consumption of styrene was only 14 (males) or 21 (females) mg/kg/day at the higher dose level. Fifty male and 70 females in each treated group and 76 male and 106 female control animals were included. At termination, organ weights were determined and histopathology carried out on a large number of tissues. The only systemic toxicity finding was a slight but significant reduction in bodyweight of 10% in females at 250 ppm after 2 years. There were no treatment-related increases in the incidences of tumours in treated groups. However, the dose levels used in this study were low, given that other studies in rats have been able to employ much higher doses, and therefore the study cannot be regarded as a rigorous examination of the carcinogenic potential of styrene.

A study in BDIV rats is available in which the animals were exposed *in utero* and then from weaning for 120 weeks (Ponomarkov and Tomatis, 1978). This is an unusual protocol for carcinogenicity testing. Pregnant animals were given a single dose of 1350 mg/kg of styrene in olive oil on day 17 of gestation, and groups of about 70 progeny per sex were then dosed with 500 mg/kg once a week until the experiment was terminated at week 120. The control group consisted of 36 male and 36 female progeny from maternal animals and received vehicle only. No effects on mean survival time or on bodyweight gain were noted. Signs of lung ad kidney damage were noted at autopsy in animals dying at 50-60 weeks, and kidney and stomach lesions were seen in those dying after 80 weeks. No significant increases in tumours of any type were noted. No evidence of styrene-induced carcinogenicity in rats is presented in this study.

Mouse

As part of the NCI (1979) study, groups of 50 B6C3F_1 mice per sex were administered 150 or 300 mg/kg of styrene by gavage, as a solution in corn oil, five days a week for 78 weeks (NCI, 1979). The animals were then observed for a further 13 weeks. Only a small control group consisting of 20 males and 20 females was used.

A significant decrease in survival was noted in the male mice at 300 mg/kg, with 78% surviving the experimental period, as compared to 92% and 100% of the animals at 150 mg/kg and in the control group respectively. A slight but not statistically significant decrease in survival was noted in the female mice, with 76% and 80% of animals of the 300 and 150 mg/kg groups respectively surviving the experimental period, compared to 90% of the controls. A “slight” dose-related decrease in bodyweight gain was noted in the female animals.

The tumour incidence was calculated on the basis of the animals surviving for at least 52 weeks. A statistically significant and dose-related increase in pulmonary tumours was noted in the male mice of both dose groups compared to controls. Alveolar or bronchiolar tumours were noted in 9/49 (18%) of males in the 300 mg/kg group, these consisted of four adenomas and five carcinomas. In males of 150 mg/kg dose group, 6/44 (14%) of the animals had lung tumours; these consisted of three adenomas and three carcinomas. No lung tumours were noted in the 20 control male mice. The incidence of pulmonary adenomas in the treated females was 3/43 (7%) and 1/43 (2%) in animals at 300 and 150 mg/kg respectively. No carcinomas were noted. No lung lesions were noted in the 20 controls. Historical control data suggested a mean spontaneous incidence of 12% for lung tumours in this strain of mice. A “slight” but not statistically significant increase in the incidence of hepatocellular adenomas was also noted in the female mice. This finding is not considered to be of toxicological significance. There were no significant differences between groups in the tumour incidences at only other sites.

Overall in this study a statistically significant increase in pulmonary tumours was noted in male mice given styrene at doses of 150 mg/kg/day and above. The value of this study is limited by the small number of control animals used. However it is important to consider that an increase in lung tumours in mice has also been reported in a 2-year inhalation study with styrene (see above). Therefore, it seems probable that styrene did produce a carcinogenic effect in the lungs of male mice in this study.

A series of studies conducted in two strains of mice (O2O and C57B1) is available in which the animals were exposed *in utero* and then from weaning for 120 weeks (Ponomarkov and Tomatis, 1978). In the first study, the inbred O2O strain was used. Pregnant animals were given a single dose of 1350 mg/kg of styrene in olive oil on day 17 of gestation and groups of about 40 progeny per sex were given the same dose once a week from weaning for 16 weeks. They were observed until week 100 when the experiment was terminated. Control groups of 20 progeny per sex from vehicle-treated animals and 54 male and 47 female untreated animals.

A marked decrease in survival was noted in the treated animals. Lesions in the liver, spleen and lungs were noted in the treated animals at autopsy. Tumour incidence was calculated on the basis of those animals surviving at the time of detection of the first tumour in decedents at necropsy. An increased incidence of pulmonary tumours (adenomas and adenocarcinomas) was observed in the treated animals. These were noted in 83% (20/23) of the males and 100% (32/32) of the females. This increase was statistically significant in both sexes as compared to the vehicle-treated controls, where the incidence was 42% (8/19) in the males and 67% (14/20) in the females. When comparison was made with the untreated controls (the incidences in these untreated control groups being 64% (35/53) in males and 53% (25/47) in females), only the female test group incidence was statistically significantly higher. Since the spontaneous incidences of these tumours are very high in this strain of

mouse, the additional tumours seen in styrene-treated animals are likely to have arisen from further enhancement of an already active, underlying cancer-generating process. The toxicological significance of this is doubtful. There was no styrene-related increase in the incidence of tumours at any other site.

In the second study, carried out with inbred C57B 1 mice using a very similar protocol and a dose level of 300 mg/kg, no significant increase in tumours of any site was observed in the treated animals. Overall, the unusual nature of these studies and the pattern of results obtained mean that they do not contribute to an understanding of the carcinogenic potential of styrene.

Dermal

No studies are available.

Other routes

In a study in which groups of 25 A/J mice received ip doses of styrene 3 times per week for around 7 weeks (total dose 200 μmol) there was no increase in the incidence of lung adenomas or adenocarcinomas compared to controls (Brunnemann *et al*, 1992).

Recent studies exploring the mechanism of styrene-induced lung tumours in mice

Kaufmann *et al*. (unpublished, 2004) investigated whether styrene oxide (SO), ring-oxidised (4-vinylphenol) and side-chain hydroxylated (phenylacetaldehyde, phenylacetic acid, 1-phenylethanol, 2- phenylethanol, acetophenone) styrene metabolites induce cell proliferation and glutathione depletion in mouse lungs. Groups of 8-10 female CD-1 mice received single doses of 0 or 100 mg/kg styrene, 100 mg/kg SO, 5, 20 or 35 mg/kg 4-vinylphenol (4-VP), 5, 15, 35 or 100 mg/kg phenylacetaldehyde (PA), 5, 15, 35 or 100 mg/kg phenylacetic acid (PAA), 100 mg/kg acetophenone, 100 mg/kg 1-phenylethanol (1-PE) or 100 mg/kg 2-phenylethanol (2-PE) on 3 consecutive days. The test article was administered by the ip. route via 3 separate injections per day over 3 days. Sub-cutaneously implanted osmotic mini-pumps were used to administer bromodeoxyuridine (BrdU) to assess S-phase DNA synthesis (a measure of cell proliferation). On completion of 3 days animals were killed and examined macroscopically. Subsequently, lungs were weighed and subjected to histopathological examination. Additional immunoistochemical staining was conducted to assess S-phase DNA synthesis and glutathione-positive cells.

Administration of styrene resulted in 4-5 fold increases in cell proliferation (as measured by BrdU labelling index) in the large/medium bronchi, terminal bronchioles and alveoli. The effects of SO were more marked than those of styrene. SO produced a 7-fold increase in cell proliferation in the large/medium bronchi and a 10-fold increase in the terminal bronchioles, but only a marginal increase (1.5 fold) in the alveoli. SO also resulted in an increased number of apoptotic cells in the large/medium bronchi. In addition, SO caused glutathione depletion in the bronchiolar epithelium. 4-VP at 60 and 105 mg/kg/day produced 13-19 fold increases in cell proliferation in the large/medium bronchi and terminal bronchioles, but there were no effects in the alveoli. 4-VP at 105 mg/kg/day also caused glutathione depletion in the bronchiolar epithelium. PA and PAA at 300 mg/kg/day produced 5-6 fold increases in cell proliferation in the alveoli, together with increased apoptosis. Both of these substances seemed to inhibit cell proliferation in the large/medium bronchi and terminal bronchioles. 1-PE and 2-PE produced only marginal (1.5 fold) increases in cell proliferation in the alveoli, and acetophenone did not produce any evidence of proliferation in any of the lung compartments examined.

Histopathologically, SO at 300 mg/kg/day and 4-VP at 105 mg/kg/day caused a flattened appearance of the bronchiolar epithelium in the large and medium bronchi and terminal bronchioles. These are considered to be the first cells repopulating the affected bronchi and bronchioles following cell necrosis. There was also a loss of the usual bulging of the apical cytoplasm of Clara cells, which suggests that the Clara cells are the primary target cells of the toxic insult. 4-VP at 60 mg/kg/day did not result in any histological changes or depletion of glutathione. Under the conditions of this study and the exposure levels used, the other styrene metabolites investigated did not affect the histological appearance.

The results of this study suggest that 4-VP and SO are the two styrene metabolites that contribute most to styrene lung toxicity, with 4-VP being of greater potency than SO or styrene itself. Only SO and 4-VP produced Clara cell cytotoxicity, glutathione depletion and significant proliferation in the terminal bronchioli of the mouse lung, the anatomic site from which the tumours seen in mice (Cruzan *et al*., 2001) arise. Treatment with PA and PAA induced slight cell proliferation in the alveolar compartment with no effects in the terminal bronchioles while 1-PE and 2-PE produced marginal increases in cell proliferation in the alveoli with acetophenone producing no changes at all. Based on these results it appears that the metabolites of the side-chain hydroxylation pathway are of minor relevance in causing pneumotoxicity in the terminal bronchioli of mouse lung. The slight changes caused by these metabolites in the alveoli are likely to represent background noise. The increased apoptosis observed with SO in the large/medium bronchi is considered to be a compensatory response associated with the induction of cell proliferation rather than the initial toxic event triggering the observed cell proliferation. The authors propose that these data indicate that the mechanism of styrene-induced tumour formation in mice may be via ring-oxidized metabolites such as 4-VP. However, it should be noted that this is a short-term study and does not address the consequences of prolonged exposure; it is uncertain whether the increased cell proliferation observed with 4-VP will be sustained leading to tumour formation. Nevertheless, it is biologically plausible to assume that 4-VP-induced cell proliferation will be sustained in response to a sustained toxic insult.

In a study conducted to investigate the role of the ring-oxidized metabolite of styrene, 4-VP in mouse lung toxicity, groups of 4-5 male CD-1 mice received a single ip injection of 0 or 100 mg/kg 4-VP (Cruzan *et al.*, 2005). Animals were killed at 12, 24, 48, 72 hrs, 4 or 6 days post-dosing and lungs lavaged; the number of cells, the amount of protein and the lactate dehydrogenase (LDH) activity present in the bronchoalveolar lavage fluid (BALF) were determined.

At 12 hours post-dosing, increased LDH activity (by approximately 2-fold compared to controls) was measured; this remained elevated until day 4 post-dosing. In addition, there was an increased number of cells which remained elevated until day 6 post-dosing (increased by around 7-20 fold compared to controls). Protein levels were significantly increased on day 3 post-dosing but not at other time points. These changes suggest that a single ip administration to CD-1 mice of 4-VP at 100 mg/kg causes lung damage.

This was followed by a repeated-exposure experiment using groups of 5 female CD-1 mice and 5 female Sprague-Dawley rats. Animals were administered 0, 2, 6, 20 or 60 mg/kg/day 4-VP by 3 daily ip injections for 14 days. Blood samples were taken on days 0, 7 and 13 for determination of serum sorbital dehydrogenase (SDH). Animals were killed after 14 days, with liver and lungs examined histologically.

There were no effects on bodyweight and no clinical signs of toxicity. Also, there were no effects on SDH activity, or liver histology. Multifocal hyperplasia (minimal to slight) was seen in the medium and terminal bronchioles of 3/5 mice exposed to 6 or 20 mg/kg/day and of all mice at 60 mg/kg/day (slight to moderate). No effects were seen in the lungs or liver of rats. This study provides evidence that 4-VP is one of the major contributors to styrene lung toxicity in mice. The absence of changes in rats at the same exposure levels used for the

mouse supports the arguments for the species specificity of the lung toxic response induced by styrene.

It has been shown that a single i.p. administration of 4-VP at dose levels up to 200 mg/kg to CD-1 mice is several times more hepatotoxic and pneumotoxic than either styrene or styrene oxide (Carlson et al., 2002a and Gadberry et al., 1996). It has also been reported that the toxicity of 4-VP (a single i.p. dose of 150 mg/kg to Sprague-Dawley rats and CD-1 mice) to rodent liver and lungs is suppressed by diethyldithiocarbamate or 5-phenyl-1-pentyne, inhibitors of CYP2E1 and CYP2F2 respectively (Carlson , 2002b), indicating that the hepatotoxicity and pneumotoxicity of 4-VP are due to a metabolite(s) and not the parent compound. No marked difference between wild-type and CYP2E1 knockout (i.e. lacking the CYP2E1 gene) mice were found in the rates of microsomal metabolism of 4-VP in either liver or lung (Vogie *et al*., 2004). When mice were administered a single i.p. dose of 100 mg/kg 4-VP, the CYP2E1 knockout mice were more susceptible to hepatotoxicity, as measured by increases in serum sorbitol dehydrogenase activity, than were the wild-type mice. There was no significant difference in pneumotoxicity between the wild-type and the knockout mice, suggesting that, as for styrene, additional cytochromes P450 are involved in the metabolism of 4-VP (Vogie *et al*., 2004).

Summary of carcinogenicity studies in animals

The carcinogenic potential of styrene has been explored in rats and mice, using the inhalation and oral routes of exposure. A carcinogenic effect of styrene towards the lung is evident in the mouse. This has been shown in a well-conducted lifetime inhalation study in CD1 mice at exposure concentrations of $\geq$ 20 ppm styrene and, somewhat less convincingly, in an oral study in mice of the $B6C3F_1$ strain. The inhalation study, which included extensive histopathological examination, showed that the tumours (prevalently adenomas) were preceded by cytotoxicity characterised by early Clara cell toxicity followed by progressive bronchiolar epithelial hyperplasia and bronchiolar-alveolar hyperplasia.

In the rat, styrene has not exhibited any clear evidence of carcinogenic potential by the inhalation or oral route. In individual studies there have been isolated findings of statistically significantly higher incidences of various particular tumour types in particular groups of styrene-treated animals, compared with the in-study controls. However, the findings have been within historical background ranges, not reproducible between studies, in some cases have not shown an upward trend with increasing dose, and have not been associated with evidence of underlying styrene-induced changes at the site in question.

On the question of the relevance of the mouse lung tumours for human health, consideration of the available toxicokinetic information and data from single and repeated inhalation exposure studies in experimental rodents suggests the following as the most plausible toxicological mechanism for the mouse lung tumours. Styrene is metabolised by cytochrome P450 enzymes in the metabolically active Clara cells (non-ciliated bronchiolar epithelial cells involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium) of the bronchiolar epithelium of the mouse, producing cytotoxic metabolites of styrene including styrene 7,8 oxide (SO) and oxidative metabolites of 4-vinylphenol (4-VP). These metabolites cause early Clara cell toxicity/death and sustained regenerative bronchiolar cell proliferation which, in turn, leads to compensatory bronchiolar epithelial hyperplasia and ultimately tumour formation. Clara cell toxicity could also be a consequence of the long term depletion of glutathione, because of conjugation with SO. Genotoxicity of SO (an EU-category 2 and IARC group 2A carcinogen) or other reactive styrene metabolites is unlikely to be involved in tumour development as minimal binding of styrene metabolites to DNA has been detected in mouse lung with no species- or tissue-specificity.

All of the key events of this postulated mode of action are less operative in the non-responsive rat (which does not develop lung tumours at exposure concentrations up to 1000 ppm) and even less operative in humans.

The number of Clara cells (being responsible for both the formation of toxic metabolites and the target for their toxic action) is very low in humans, even less than in rats. While Clara cells comprise about 85% of bronchiolar epithelium in mice and 25% in rats, in humans such cells are rare.

Although the enzymes CYP2E1 and CYP2F2 required for the formation of the Clara cell toxicants such as SO (including the highly pneumotoxic R-enantiomer) and the downstream metabolites of 4-VP have been detected in human lung, their activities are low (at least 400 times lower than in the mouse) and metabolic activation of styrene to SO is minimal or undetectable.

In human lung, detoxification of SO (if formed at all in human pulmonary tissue) takes place predominantly *via* epoxide hydrolase (located on the endoplasmatic reticulum in close proximity to the toxifying cytochrome P450s). The close proximity of the "detoxifying" enzymes to any "toxifying" enzymes ensures the efficient removal of any toxic metabolites. Rodents use both epoxide hydrolase and glutathione-S-transferase as detoxification pathways with the mouse relying on glutathione conjugation more so than the rat. As glutathione S-transferase is located in the cytosol, this makes this detoxification pathway less efficient than the epoxide hydrolase pathway. In comparison to the rodent species, in humans, SO detoxification proceeds nearly exclusively via epoxide hydrolase and glutathione S-transferase accounts for only 0.1% of SO detoxification.

Taking account both of the toxification to SO and its detoxification, PBPK-modelling has shown that the SO content of human lungs must be very small, if there is any.

Formation of 4-VP and its downstream metabolites occurs at a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse concentrations). Although it cannot be ascertained whether or not these species differences in the formation of 4-VP metabolites in the lung may be a reflection of the different numbers of Clara cells (the metabolically active lung cells) present in the different species, since 4-VP metabolites are produced by the same cytochrome P450 enzymes involved in the production of SO, it is most likely that the species differences in the formation of 4-VP metabolites observed reflect species differences in metabolic capability.

As indicated by PBPK-modelling, glutathione depletion caused by SO does not occur in humans. Also, as reactive downstream metabolites of 4-VP are formed in human lung only to a very small extent, the 4-VP metabolic pathway is not expected to cause any glutathione depletion in human pulmonary tissue.

There is no evidence from extensive epidemiological investigations that long term exposure to styrene has produced lung damage or lung cancer in humans (see sections 4.1.2.6.2 and 4.1.2.8.2).

Hence, overall, the weight of evidence appears to indicate that the consequences of long term exposure to styrene in mouse lung cannot be replicated in the human situation at relevant levels of exposure. Although there are still some uncertainties in this postulated mode of action and in its relevance to humans, namely the lack of data on the relative rates of 4-VP metabolites detoxification in different species, no alternative modes of action that logically present themselves can be supported by as significant a body of evidence as the one presented in this assessment. Consequently, it is felt that the level of confidence in the postulated mode of action can be reasonably high and that, in view of the extensive negative lung epidemiology (see section 4.1.2.8.2), it is reasonable to conclude that the lung tumours seen in mice are unlikely to be of any relevance for human health. A more detailed analysis (according to the IPCS framework for evaluating a mode of action in chemical carcinogenesis) of the evidence in support of the proposed mode of action and of its

relevance for human health is presented in Annex A to this document. Whether this mechanism could be operative in humans at other sites cannot be excluded. However, it is noted that several cohort and case-control studies of workers exposed to styrene have shown no evidence for a causative association between styrene exposure and cancer in humans at any site (see section 4.1.2.8.2) and no consistent evidence for styrene-induced toxicity in any organ has emerged from studies of exposed workers (see section 4.1.2.6.2).

4.1.2.8.2 Studies in humans

A number of mortality studies are available. These concern workers exposed to styrene in styrene monomer production, styrene polymerisation, styrene-butadiene rubber (SBR) production and glass-reinforced plastic manufacture. Mixed exposure to other chemicals can occur in these industries; this is particularly true of styrene-butadiene rubber production plants. However, relatively high exposure to styrene in the absence of significant exposure to other chemicals occurs in the glass reinforced plastics (GRP) industry.

Recently, the potential association between environmental exposure to styrene and to other chemicals and the incidence of breast cancer has been investigated in an ecological study.

Glass-reinforced plastics (GRP) industry

A number of studies have investigated whether or not exposure to styrene is associated with cancers of the lymphatic and haematopoietic system (LHS) because of observations of increased risk for these cancer types particularly among workers in the SBR industry. However, in the GRP industry, the hypothesis that exposure to styrene is linked with LHS (or any other) cancer is difficult to test epidemiologically because it is usual for only a relatively small number of workers in a population to remain in an exposed job for more than 2 years.

A large, eight-centre cohort mortality study was initiated by IARC (WHO) and the Commission of the European Communities and covered analysis of data on GRP workers in Denmark, Finland, Italy, Norway, Sweden and UK (Kogevinas *et al*, 1993, 1994a, b). The individual cohort numbers varied from 1,438 to 15,867 with varying follow-up periods lying between 1945 and 1991. The average follow-up was 13 years. Of a total of 41,167 potential candidates for the cohort, 479 were excluded because of a lack of data. Those lost to follow-up were 1.4% and a further 1.6% emigrated and were not subsequently traced. About 60% of individuals in the study (24,794) were employed for less than two years in GRP manufacture; this is common for the industry. Of workers employed for more than 2 years, about 11,500 were followed up for more than 10 years. Some personal airborne exposure data (about 16,500 measurements) and urine metabolite data (about 18,500 measurements) were available, but not consistently for the whole exposure period. Extensive exposure data for the early period of the study (pre-1970, when average exposures were relatively high) were available only for one country's cohort included in the study. Therefore exposure estimates for the early period (pre-1970) for the other countries were derived by modelling. Two exposure models were used; in A, extrapolation to the earliest measurements (found in the Danish cohort) was carried out; in B the earliest data for the cohort were assumed to apply to the earlier part of the individual study. For some countries most of the exposure measurements were short-term measurements (< 2 hours sampling time) or spot samples, therefore 8-hour TWA exposure values were estimated from these. Cumulative exposure (ppm-years) was calculated. Different job exposure categories were used to subdivide the workers. The exposed subgroups were "laminators" (n=10,629, "unspecified" (n=19,408) and "other exposed" (n=5,406); unexposed subgroups were "not exposed" (n=4,044) and "unknown job title" (n=1,201). Mean 8-hour TWA exposure for each of the exposed subgroups, based on measurement for five countries and after adjustment for sampling time,

were 46 ppm for laminators, 28 ppm for the “unspecified” exposure category and 15 ppm for the ‘other exposed’ category. A comparison was made with national statistic for the calculation of standardised mortality ratios (SMRs). IARC converted between the 7th and 9th revisions of ICD to allow pooling of results. Regression models were fitted to country, age, sex, time and exposure specific rates.

Mortality from all causes was lower than expected, an apparent healthy worker effect (SMR 0.92, 95% CI 0.88-0.95, 2714 deaths). There were no significant excess mortality results for specific types of cancer. Death rates for LHS cancer in exposed workers were not statistically increased (SMR 0.98, 95% CI 0.72-1.30, 49 deaths). In workers with 20 years or more since first exposed, the SMR for LHS cancer was 1.32 (10 deaths, 95% CI 0.64-2.44). The linear trend for years since first exposed and LHS cancer was statistically significant. A similar pattern of results was found for non-Hodgkin’s lymphoma, Hodgkin’s disease and leukaemia. However, there was no association between LHS cancer mortality and duration of exposure; workers who had been exposed for less than 2 years generally had higher SMRs than those with $\geq$ years’ exposure. Increasing cumulative exposure to styrene did not relate positively to increasing LHS cancer death rate, although increasing average exposure level was related to increasing LHS cancer rate ratios. Both the average and the cumulative exposures are based on the exposure models and therefore might be subject to errors arising in the models. The association of SMR with time since first exposed and with average exposure concentration is not supported by an association with cumulative (i.e. total) exposure. The only other notable findings were a slight increase in rate ratio (RR) for pancreatic cancer and oesophageal cancer with increasing cumulative exposure; for both these cancers, RRs were highest in workers with $\geq$ 20 years since first exposure. However, in each case, the findings were not statistically significant and are not considered to provide evidence of an association with styrene exposure. Overall, the results do not provide convincing evidence of styrene possessing carcinogenic potential in humans.

Wong (1990) and Wong *et al* (1994) have reported a study with a cohort of 15,908 workers at 30 GRP plants in the United States. Individuals were entered in the study if they had worked in styrene-exposed jobs for a minimum of 6 months between 1 January 1948 and 31 December 1977. The update (Wong *et al*, 1994) was an extension to 31 December 1989. Almost half of the cohort (46%) had worked for less than 2 years and about a third (32%) had worked for 5 years or more in the GRP plants. Social security and other national sources were consulted to determine vital status and death certificates were obtained. Standard mortality ratios were calculated from age, sex, race and calendar year-specific US death rates. However, no race information was obtained for most of the cohort and therefore it was assumed that the cohort was white. Smoking data were not collected. Data on work practice and past occupational hygiene measurements were used to develop a job exposure matrix and to determine 8-hour TWA exposure levels. Different job categories involved a range of exposure levels of 1-200 ppm 8-hour TWA. Job histories were available for cohort members and therefore exposure levels and cumulative exposures were determined in the first study. In the update, some duplicate records were identified, and 52 workers were found to have had less than 6 months experience; these data were excluded from the updated analysis. The final cohort size was 15,826. Death certificates were obtained for 1,586 decedents (97.4% of the total number of decedents). Vital status was determined for 96.5% of the cohort.

Overall mortality (SMR 1.08, 95% CI 1.030-1.13) and total deaths from cancer (SMR 1.16, 95% CI 1.05-1.27) were slightly increased. Significant increases were observed in some cause-specific death rates. For cancer of the oesophagus, the SMR was 1.92 (14 deaths, 95% CI 1.05-3.22), for cancer of the lung the SMR was 1.41 (162 deaths, 95% CI 1.20-1.64), for cancer of the cervix and uteri the SMR was 2.84 (10 deaths, CI 1.36-5.21) and for other female genital organ cancers, the SMR was 2.02 (13 deaths, 95% CI 1.08-3.45). The number of observed deaths was lower than expected for LHS cancers. The highest SMRs for all cancers, lung cancer and cervix and uteri cancer were found in the workers who had

been employed for no more than one year in the GRP industry, and therefore are not related to styrene exposure. Furthermore, for lung cancer in particular, given the lack of data on tobacco smoking, the increased SMR observed cannot be clearly attributed to styrene exposure. There was no trend for SMRs to increase with duration of employment; for many causes the opposite occurred. For almost all the cohort (98%), duration of employment was the same as duration of exposure to styrene. The lowest cumulative exposure group had the highest number of significant cause-specific excesses in mortality. Increases in mortality in workers employed for only a few years are more likely to be related to socio-economic factors associated with short-term working. This general consideration, together with the pattern of results obtained suggests that overall, there was no evidence of a causative association between increased cancer mortality and exposure to styrene in this study.

As part of the larger IARC study (discussed above), the mortality experience of a cohort of GRP workers in the UK was investigated (Coggon *et al*, 1987). The cohort consisted of 7949 workers employed between 1947 and 1984 at 8 facilities. The main part of the analysis was restricted to 7 plants where the proportion of the cohort that could be traced was satisfactory (96.7%). Laminating jobs, where styrene exposure was highest, involved exposure to 8-hour TWA levels of 40-100 ppm styrene. Overall, mortality was less than in the general population (SMR 0.83, 95% CI 0.77-0.89). Although excess deaths from lung cancer (SMR 1.12, 95% CI 0.89-1.39, 83 deaths) were found, information on smoking habits was not available. The risk of lung cancer deaths was not statistically significantly increased and did not increase with time since first exposure. This cohort with follow-up to 1990 was entered in the international IARC study outlined above (Kogevinas *et al*, 1993, 1994a, b). Within the extended follow-up, the increase in lung cancer had decreased (SMR 1.06, 95% CI 0.84-1.32, 77 deaths). Therefore the excess is not considered to be attributable to styrene exposure. There were no other statistically significant increases in death from particular cancers. Deaths from cancer of the lymphatic and haematopoietic systems (LHS) were not increased (6 observed, 14.9 expected). Overall, no evidence of styrene exposure inducing increased cancer mortality was apparent in this study.

A group of 12,800 Danish workers from the IARC study discussed above were included in a study of LHS cancer incidence (Kolstad *et al*, 1994). All companies producing GRP in Denmark between the early 1960s and 1988 were traced. In total, 36,525 male workers were thought by dealers in raw materials to have been exposed to styrene, although it is possible that some may not have been exposed. This group included 60% who were employed for less than one year. Employers were also questioned and they identified only 28,518 workers exposed. The overlap agreed to by both sources was 26,784 but the dealers' estimate was used in the analysis; no explanation for this choice was offered. The authors also outlined another estimate that gave only 15,550 exposed workers. Therefore, the accuracy of the number of exposed workers used in the calculations of standardised incidence ratios (SIRs) was highly questionable. SIRs were calculated using national standardised rates. In the cohort, the incidence of LHS cancer was not significantly increased; SIR 1.20, 95% CI 0.98-1.44, 112 cases, 93.7 expected. Leukaemia SIR was 1.57 (95% CI 1.07-2.22) in workers with more than 10 years since first employment. However, the excess in this group was specifically due to leukaemia cases among workers with less than one year of employment. The very short period of styrene exposure casts doubt on any causative association between styrene exposure and these leukaemia. There were no other significant increases in SIR for site-specific cancers. Given the uncertainty surrounding the true number of styrene-exposed workers in this study, firm conclusions cannot be drawn from the results, although there was no evidence of any carcinogenic potential for styrene.

Mortality from "solid" cancers in a cohort of 36,610 styrene-exposed workers employed between 1964 and 1988 at 386 facilities in Denmark has been investigated (Kolstad *et al*, 1995). A reference population of 14,293 non-exposed workers in the same plants, was also investigated. No details of medical histories or smoking habits were reported. No information

regarding potential exposures to other chemicals was reported. No data on individual exposures or job titles were available. Employees of a company with <50% of the workforce involved in work with reinforced plastics were classified as having probable low styrene exposure, whereas employees of companies with >50% of the workforce working with reinforced plastics were classified as having probable high styrene exposures. Duration of employment was determined from payment records.

Overall mortality was increased by 10% compared with Danish national rates (SMR 1.10, 95% CI 1.06-1.14, 3031 deaths). Of these deaths, there was no evidence of increased mortality due to 'solid' cancers (SMR 0.99, 95% CI 0.93-1.05, 1134 deaths). Mortality rates in non-exposed workers were comparable to national rates. A comparison of SMRs for site-specific 'solid' cancers was made between exposed and non-exposed workers. A number of specific types of cancer showed higher SMRS in the exposed workers compared with unexposed workers. However, none of the SMRs in exposed workers were statistically significant. Overall, there is no evidence from this study that styrene exposure induces an increased cancer risk.

A cohort in Central Bohemia consisting of 215 individuals employed in manual lamination processes (72% women) in 5 plants between 1970 and 1988, was investigated in relation to the incidence of neoplastic diseases (Znojemska and Svandova, 1991). The minimum duration of employment was 5 years (mean working period 10.7 years). Styrene exposure was reported to be 66 ppm (8-hour TWA), with peak exposures of 439 ppm. The majority of the cohort was reported to be non-smoking. Information concerning worker health was obtained from medical records. The cohort did not include styrene-butadiene rubber manufacture workers. The observed incidences of cancer and mortality were compared with Czech Republic national data. Overall, 13 tumours were observed in the workers, of which 5 were terminal. The expected incidence was 13.2, with 7.9 terminal instances. When females were analysed separately, 12 instances of tumour development were observed (9.8 expected), with 5 reported deaths (expected 5.5). Overall, no evidence of styrene exposure including increased cancer mortality was apparent in this study.

Mortality of a cohort from two US plants was studied (Okun *et al*, 1985). Workers employed in the facilities for at least one day between 1959 and 1978 were eligible for entry into the cohort; a total of 5,201 workers representing 96% of those eligible had complete records and entered the study. Jobs were classified according to occupational hygiene surveys, and higher and minimal styrene exposure subgroups were defined. In the higher exposure subgroup, there were 2,060 individuals but of these 73% had worked in the "high" exposure job for one year or less. The death rates in the cohort were compared with those of the general US population. Death certificates were obtained for 94% of the deceased. The number of deaths in the cohort was 176 (SMR 0.90, 95% CI not specified). In the higher exposure subgroup, the number of deaths was 47 (SMR 1.13, 95% CI 0.99-1.80). There were no excess deaths due to malignant neoplasm in this subgroup (SMR 0.86, 95% CI 0.23-2.21) and no lymphoma or leukaemia deaths were found in this subgroup or in the total cohort. In the minimal exposure subgroup, 127 deaths occurred (SMR 0.85, 95% CI not specified) and the SMR for malignant neoplasm was 1.11, 95% CI not specified (32 deaths). Overall, there was no evidence of styrene carcinogenicity in this study, although it had relatively little power to detect mortality due to exposure to styrene since only 39% of the higher exposure group were observed for more than 10 years from the date of first employment in the styrene-exposed job.

Ruder *et al*, 2004 have produced a 21-year follow-up of earlier work (Okun *et al*, 1985). There were slight increases in the rate of death due to malignant neoplasms in the high-exposure (n=58, SMR 1.26, CI 0.96-1.63) and low-exposure (n=175, SMR 1.14, CI 0.98-1.32) groups. There was no increase in deaths due to leukaemia or lymphoma although increased SMRs were observed for some cancer sites that were not anticipated to be related

to styrene exposure: oesophageal cancer (n=12, SMR 2.30, CI 1.19-4.02), prostate cancer (n=24, SMR 1.71, CI 1.09-2.54), urinary tract (n=6, SMR 3.44, CI 1.26-7.50). The increased risks of death due to oesophageal and prostate cancer were not exposure-related, and in the high exposure groups the confidence intervals were relatively large and notably below 1.0 casting some doubts on the significance of the apparent association with styrene. The increased risk of death due to urinary tract (kidney and bladder) cancer was seen in the high exposure group but not low exposure but is not consistent with other epidemiology studies; the authors conclude that it may have arisen due to chance.

Overall, the results of this study do not show a consistent or clear pattern of a causative association between increases in any cancer type and styrene exposure.

Overall the results from several major epidemiological studies available in GRP workers show no consistent or clear evidence of a causative association between increases in any cancer type and styrene exposure. The increased risks for lymphatic and haematopoietic neoplasms observed in some of the studies are generally small, statistically unstable and often based on subgroup analyses. These findings are not very robust and the possibility that the observations are the results of chance, bias or confounding by other occupational exposures cannot be ruled out.

Styrene and styrene products production

The mortality of 622 male workers exposed to styrene and of 3072 males 'unexposed' to styrene, who were employed for at least 1 year during the period of 1945-1974 at a single UK site producing, polymerising and processing styrene, was surveyed up to the end of 1978 (Hodgson and Jones, 1985). Particular consideration was given to mortality from lymphoma and leukaemia. No measured levels of styrene exposure at the site and no information regarding potential exposure levels of other chemicals were available. The exposed population included 131 men who were potentially exposed to styrene and other chemicals in the laboratory. The remaining 491 workers were manual workers having mixed chemical exposures, but with specific potential exposure to styrene. No information on individual smoking habits or behavioural factors was available. No details of any questioning with respect to previous radiographic or chemotherapeutic treatments were reported. Mortality information was obtained for all except for 14 emigrants in the exposed population and 181 in the reference population. Mortality rates were compared with appropriate national rates, and where available, with regional rates. SMRs were calculated only when $\geq$10 deaths were observed; all SMRs were rounded, to reflect the wide confidence intervals.

SMRs for all causes of death and from all malignant neoplasm were similar in exposed workers and in the reference population, and were lower than expected compared with national rates. There were no deaths from leukaemia in either the exposed or the reference populations, although one exposed individual had leukaemia noted on the death certificate (not as cause of death). This individual was included in subsequent analyses of deaths from lymphatic and haematopoietic cancer. The only statistically significant excess of mortality was from lymphoma in the exposed population (3 deaths compared with 0.56 expected; SMR not calculated); no excess mortality from this cancer type was observed in the reference population (3 observed, 3.02 expected; SMR not calculated).

In the absence of information on actual levels of styrene exposure, length of service was used as a surrogate, to determine any possible relationship between styrene exposure and death from lymphatic and haematopoietic cancer for the four cases (3 lymphoma, 1 leukaemia). There was no association found between length of service and the incidence of lymphatic and haematopoietic cancer in exposed workers. For two of the cases, each under 40 years old at death, length of service was 1-2 years. This very short period of styrene exposure casts doubt that there is any causative association between styrene exposure and

these deaths. In addition, each of the four cases had potentially confounding exposures, such as benzene and ethylene oxide. Overall, given the small number of deaths in this study, the absence of any apparent relationship between length of exposure and death, and the presence of confounding factors, the apparent finding of excess mortality from lymphatic and haematopoietic cancer is not considered to provide any reliable evidence of an effect of styrene exposure.

A cohort with a total of 2,904 workers having at least one year of employment in four US styrene plants has been studied (Ott *et al*, 1980). Some styrene monomer production, research and some styrene polymerisation, including a very small section of styrene-butadiene manufacture (see later in section), took place at the sites where cohort members worked. Workers were exposed to a variety of chemicals including styrene, benzene, alkylbenzenes, cadmium and chlorinated solvents. Exposure was defined by job category and job histories. Mortality was followed from 1940 to 1975. The "all causes" SMR compared against the US population, was 0.71 (303 deaths); total deaths from malignant neoplasm had an SMR of 0.76 (58 deaths observed), an illustration of the healthy worker effect. Non-statistically significant increases in the numbers of deaths from LHS cancer (SMR 1.32, 95% CI 0.58-2.72) and leukaemia (SMR 1.76, 95% CI 0.64-3.83) were observed. Overall, there was no evidence for styrene-induced carcinogenicity in this study.

A follow-up of this cohort was reported (Bond *et al*, 1992). An extra 11 years of follow-up was available in the updated study; vital status was ascertained at 1 January 1987 and exposure categories were re-assessed. The number of deaths was 687; 9 workers were lost to follow-up. Comparing against the US population, the all-causes SMR was 0.76 and all cancers SMR was 0.81. There was no statistically significantly elevated death rates for any specific cause (LHS cancers SMR 1.44, CI 0.95-2.08, 28 deaths). The same pattern of results was seen in comparison with company employees in the same area but statistical significance was reached for multiple myeloma (rate ratio 2.45, 95% CI 1.07-5.65). A non-statistically significant excess mortality from LHS cancer was observed in the 'styrene polymerisation and colouring' category (SMR 1.72, 95% CI not reported, 16 deaths). The excess LHS deaths were mainly in professional workers (SMR 1.77, 95% CI 0.71-3.65, 7 deaths) and production workers exposed to extrusion fumes and colorants (SMR 2.63, 95% CI 1.20-5.00, 9 deaths). However, there was no pattern of increased death rate from LHS cancers at higher estimated styrene exposures or with duration of exposure. The overall pattern of results from this cohort provides no convincing evidence of a causative association between styrene exposure and an increase in any specific cancers.

A mortality survey of 560 individuals who were employed on 1 May 1960 and with at least 5 years of employment in a US plant manufacturing styrene and polystyrene is available (Nicholson *et al*, 1978). Exposed workers were classified as being in a 'high' styrene exposure group (5-20 ppm, n=444) or in a 'low' styrene exposure group (<1 ppm, n=116) based on their job description. No information is available on whether or not the exposure data represent personal exposures, nor the reference period to which they refer. Workers were also reported to be exposed to a variety of other chemicals including benzene and ethylbenzene, although no details regarding possible levels of exposure are reported. All individuals were traced to 1975 and their vital status determined. Expected deaths in the US general population and observed deaths in workers, by cause, were determined from 1 May 1960, or from the 10th anniversary of employment, up to 31 December 1975.

At study termination 233 cohort members were still employed at the facility, 241 had retired or terminated employment and 83 had died. The level of decedents was comparable with the expected frequency in the general population SMR 1.06). There were no increases in the incidence of deaths due to cancer of the lung, leukaemia, lymphoma, "other cancers", cardiovascular disease, respiratory disease or other causes, observed in exposed workers compared with the general population.

A mortality survey of 1960 past and present workers having at least one month of employment in the German styrene or polystyrene manufacturing industry since 1931 was conducted (Frentzel-Beyme *et al*, 1978). The date exposure started and ceased, the reason for leaving and the date of birth of each employee were obtained from records. Employees were then followed up as much as possible (96% follow-up for German nationals, but only 29% for non-German nationals). Data regarding atmospheric styrene exposure levels and possible co-exposures to other chemicals are not reported in this paper. From the records available, the authors were unable to determine the length of an individual's exposure to differing levels of styrene. Therefore, given that improved control systems had been introduced, the cohort was divided in to those employed pre-1960 and those employed post-1960, as a crude means of differentiating between high and low exposure levels. For data analysis the cohort was further subdivided based on the duration of employment (<5 years, 5-10 years, 10-15 years and >15 years) and on the age of the individual.

Seventy-four (74) deaths were reported in the styrene-exposed workers, with the majority of these workers being in the age range 55-64 years. This frequency was reported to be comparable with the frequency expected in the general population (data not presented). When the relative frequency of the cause of death was compared with the corresponding distribution for the general population, no styrene exposure-related increase was evident in the number of deaths due to malignant neoplasm, cardiovascular disease or any other cause.

In a Canadian case-control study, 3,730 cancer patients and two groups of 533 control individuals in Montreal were interviewed and job histories between 1979 and 1986 were recorded (Gerin *et al*, 1998). Patients with 15 types of cancer were included in the study, including non-Hodgkin's lymphoma, Hodgkin's lymphoma, rectal, prostrate and lung cancer. Patients with leukaemia were excluded from the analysis; however the report does not state the reason for this exclusion. Job histories were translated by a "team of experts" into qualitative occupational exposure groups, with respect to potential exposures to benzene, toluene, xylene and styrene. For most subjects, exposure levels were believed to be "low" but no quantitative exposure data were reported. For most cancer sites there was no evidence of increased risk due to styrene exposure. The only exceptions were with respect to "medium to high" styrene exposures, for which an increased risk of cancer of the rectum (5 such cancer patients believed to be exposed to styrene OR 5.1 95% CI 1.4-19.4 compared with 4 controls; OR 1.0 95% CI not calculated) and cancer of the prostrate (7 such cancer patients believed to be exposed to styrene; OR 5.5 95% CI 1.4-21.8 compared with 3 controls; OR 1.0 95% CI not calculated). However associations with cancer at these sites have not been found in any other cancer epidemiology study on styrene, and in view of this and the case-control nature of the study, these apparent associations are considered to be chance findings.

In another case-control study, mortality records from 1984 to 1989 from 24 US states were analysed to determine if any associations between occupational exposures and breast cancer risk could be identified (Cantor *et al*, 1995). Cases for analysis were women whose death certificates cited breast cancer as the underlying cause of death. A total of 59,515 female deaths were reported as being due to breast cancer. Four control subjects per cancer case were randomly selected from all non-cancer deaths and were matched for age, gender and race. Individuals were coded for occupation and industry of employment (further details not reported). Women were grouped qualitatively with respect to probable exposure levels details not reported), but no quantitative data were available. Those individuals whose occupation was classified as 'homemaker' were excluded from the cohort. The final cohort for analysis consisted of 29,397 white and 4,112 black women with breast cancer and 102,995 white female and 14,839 black female controls.

Slightly higher odds ratios (Ors) were observed for exposure to styrene. The results were as follows: probable low exposure to styrene, 804 cases of breast cancer amongst white women, OR 1.13, 95% CI 1-1.2; 80 cases amongst black women, OR 1.5, 95% CI 1-1.2; probable low to medium levels of styrene exposure, 527 cases amongst white women, OR 1.18, 95% CI 1-1.3; 61 cases amongst black women, OR 1.52 95% CI 1-2.1; probable medium to high levels of styrene exposure, 64 cases amongst white women, OR 1.38, 95% CI 1-1.9; 7 cases amongst black women, OR 1.32, 95% CI 0.5-3.3; probable high levels of styrene exposure, 4 cases amongst white women, 3 cases amongst black women (the low numbers prevented meaningful statistical analysis for this latter group of women). Increased incidences of breast cancer were also reported with other exposure scenarios analysed, i.e. exposure to paints, metal/metal oxides, lead, cadmium, etc. None of the odds ratio values had a statistical significance above the 5% level, there was no evidence of a dose-response trend, and other studies have not identified elevated incidences of breast cancer among styrene-related carcinogenic action.

An abstract of a study is available in which the cancer incidence among Finnish workers exposed to the aromatic hydrocarbons, styrene, toluene and xylene has been investigated (Anttila et al, 1998). The cohort comprised 3,922 males and 1,379 females occupationally exposed between 1973 and 1992. Personal monitoring of exposure was conducted; however no data are presented in the abstract available. The overall rates of cancer incidence for the total cohort were comparable to those for the general population. For styrene exposure specifically, there was a statistically significantly increased risk for rectal cancer (SIR 3.11, CI 1.14-6.77), and non-significantly elevated risks for pancreatic and nervous system tumours. However, given that this study is reported in abstract form only, no reliance can be placed on the results.

Overall the data from several studies available in workers employed in styrene and styrene product production, have not produced any consistent and clear evidence of a causative association between styrene exposure and increases in any cancer type.

Styrene-butadiene rubber

Mortality studies have been conducted on workers exposed to styrene during styrene-butadiene rubber (SBR) production. In these plants, multiple chemical exposures are common and this makes interpretation of the results more difficult. An additional complication in these studies is that many employees move between plants, and have worked in associated industries. The following studies are presented in this document for completeness as the workers have been exposed to styrene. However it is now established that the lymphohaematopoietic cancer issue emerging is related to exposure to 1,3-butadiene and not to styrene exposure; no causal relationship with styrene exposure has been demonstrated.

A number of cohort mortality studies of workers employed in the SBR industry within the USA and Canada have been reported. However, the cohorts for these investigations are drawn from a limited number of SBR plants, and therefore there is considerable overlap between study populations reported for different studies. Additionally, a number of updates of previously studied cohorts have been reported.

Two large cohorts of SBR workers have been studied. The first cohort is drawn from workers employed at two plants at Port Neches, USA, first reported by Meinhardt *et al* (1978) and updated in 1982 (Meinhardt *et al*, 1982). The second cohort is drawn from eight SBR plants within USA and Canada, excluding those at Port Neches, first reported by Matanoski *et al* (1987) and subsequently updated (Matanoski *et al*, 1990). The most recent and by far the biggest study largely combines these two cohorts, although it excludes workers at one of the

eight plants studies by Matanoski *et al* (Delzell *et al*, 1996). Although the overlap with the two previous study cohorts is not known exactly, it is expected to be large.

A study has been conducted in workers employed at two SBR plants at Port Neches, Texas (Meinhardt *et al*, 1978, 1982). Data for each plant were considered separately. Butadiene (mean concentration 1.24 ppm, range 0.11-4.17 ppm), styrene (mean 0.94 ppm, range 0.03-6.46 ppm) and benzene (mean 0.10 ppm, range 0.08-0.14 ppm) were present in the atmosphere of the first plant (plant A) in 1976. Exposure levels previous to this were not available. In this plant, employment records were available from 1943, the date when the plant opened. A total of 3494 people had been employed, 1662 of whom were white males with at least six months' employment. Vital status of the 1662 white male workers was determined at 31 March 1976; 1356 (81.6%) were identified as alive, 252 (15.2%) as dead and 54 (3.3%) could not be definitely identified, and were assumed to be alive. Deaths were classified in accordance with the International Lists of Disease and Causes of Death current at the time of death, and the classification was converted to that of the 7th revision. Age, calendar year and cause-specific mortality rates for the white male US population were used to calculate the expected number of deaths.

The SMR for all causes of death at plant A was 80. An elevated SMR value was obtained for LHS malignant neoplasms (SMR = 1.55, CI 0.71-2.94, 9 deaths), due to increased numbers of deaths from lymphosarcomas and reticulosarcomas (SMR = 1.81, CI: 0.37-5.29, 3 deaths) and leukaemia (SMR = 2.03, CI: 0.66-4.74, 5 deaths). However, small numbers were involved and none of these excesses was statistically significant.

All of the deaths from leukaemia occurred in employees who had at least six months' potential exposure between January 1943 and December 1945, a period in which a hot-temperature batch process, subsequently discarded, but which would possibly have resulted in higher exposures, was used in the plant. When the mortality of a sub-group of workers employed for at least six months between January 1943 and the end of December 1945 was examined, overall mortality was still low (SMR = 0.83, CI: 0.72-0.95, 201 deaths). Mortality due to lymphosarcoma and reticulosarcoma (SMR = 2.12, CI: 0.97-4.02, 9 deaths) and leukaemia (SMR = 2.78, CI: 0.90-6.49, 5 deaths) were increased, but again not statistically significantly.

Only butadiene and styrene airborne levels were measured at plant B. Butadiene levels (mean 13.5 ppm, range 0.34-175 ppm) were proportionately higher than styrene levels (mean 1.99 ppm, range 0.05-12.3 ppm). Employee records were available only from 1950; the plant was previously operated by a different company (1943-1947) and was shut down from 1947 to 1950. Of a total of 2015 employees, 1094 were white males with at least six months' employment; 980 (89.6%) were alive at the end of March 1976, 80 (7.3%) were known to have died and the vital status of 34 (3.1%) was unknown. The data for cause of death were analysed in the same way as for plant A. Overall mortality was low (SMR = 0.66, CI: 0.52-0.82, 80 deaths, healthy worker effect). High SMR values were seen for malignant neoplasms of the testis (SMR = 2.15, CI: 0.26-7.77, 2 deaths) and for lymphosarcoma and reticulosarcoma (SMR = 1.32, CI: 0.03-7.36, 1 death). Clearly, with the small numbers of deaths none of the results were statistically significant. No useful conclusions can be drawn from these data for plant B.

In conclusion, there is an indication of an excess of LHS cancers in workers at one styrene-butadiene rubber production facility (plant A). This excess was highest in a subgroup of employees with possibly the highest exposures to butadiene and styrene. Although there is mixed exposure in this case, the study supports a possible link between exposure to butadiene and the incidence of LHS cancer in the context of other studies. The study does not provide any evidence of a causative association between styrene exposure and cancer.

A large mortality study, conducted on behalf of the International Institute of Synthetic Rubber Producers, was performed using data from eight butadiene-styrene rubber production plants,

seven in the USA and one in Canada (Matanoski *et al*, 1982; Matanoski and Schwartz, 1987) and a follow-up was subsequently published (Matanoski *et al*, 1990, 1993). No exposure data were available but a qualitative estimate of exposure was made from consideration of job type.

Production at seven of the plants first started in 1943; production at the eighth plant started in 1957. However, record keeping at several plants was inadequate in earlier years, thus only individuals present at the time of the start of complete record keeping were entered into the study cohort i.e. 1943 for four plants and 1953, 1958, 1964 and 1970 respectively for the other four plants. In total, 13 422 workers were included in the final study. The study cohort comprised all male employees who had worked for at least 1 year and were hired after the start of production in the plant or who had worked at any time after record keeping was complete, up until the end of 1976. Death certificates were obtained for 97.2% of the 2441 decedents. All deaths were classified in accordance with ICD8. Workers whose vital status could not be traced were assumed to be alive. Standardised ratios were calculated with reference to the US male population. The data were corrected for age, race and calendar year of death. No quantitative exposure data were available. Job categories were defined and workers were coded according to these, but no attempt was made to group the jobs according to predicted exposure.

All-cause mortality was low (SMR = 0.81, CI: 0.78-0.85), as was all-cancer mortality (SMR = 0.85, CI: 0.78-0.93). A statistically significant excess of arteriosclerotic heart disease was observed in black workers (SMR = 1.48, CI: 1.23-1.76, 1.25 deaths). There was no statistically significant increase of any specific cancer type. The SMR for LHS cancers was 0.97 (CI: 0.73-1.26, 55 deaths). Within this cancer group, slightly elevated SMRs were recorded for Hodgkin's lymphoma, (SMR – 1.20, CI: 0.52-2.37, 8 deaths) and for other lymphatic cancers excluding leukaemia or lymphosarcoma (SMR = 1.11, CI: 0.64-1.77, 17 deaths). Again, these figures were not statistically significant. There was also no trend indicative of an association between length of employment and cancer mortality.

The authors related specific causes of death to job category. In production workers, who might be assumed to have had greater exposure to the styrene and butadiene monomers, a slight excess of LHS cancers was found (SMR = 1.46, CI: 0.88-2.27, 19 deaths), but this was not statistically significant. This excess was mainly due to a particularly high incidence among black production workers (SMR = 5.07, CI: 1.97-11.07, 6 deaths) which was statistically significant. The only LHS cancer type that was statistically significantly higher in all production workers was for 'other' lymphatic cancers – Non-Hodgkin's lymphoma and multiple myeloma (SMR = 2.60, CI: 1.19-4.94, 9 deaths). Black production workers had a statistically significant excess of leukaemias (SMR = 6.56, CI: 1.35-19.06, 3 deaths). In comparison, maintenance workers, who are expected to have some incidental exposure to styrene-butadiene, showed no excess of LHS cancers or of any other specific cancer. It is possible, however, that at least up to the late 1960s, black production workers may have had higher average exposures than white production workers due to job segregation by race (Landrigan, 1993).

Overall, this study provides some evidence for an excess of LHS cancers in styrene-butadiene workers at these plants. The only significant excess of these cancers appears in a sub-group of workers involved in production jobs. Production workers are predicted to have had the greatest exposure to the styrene and butadiene monomers. However, the excess was concentrated among black workers, and not distributed evenly among the population. This may represent an artefact, particularly since when racial status was unknown for any worker, the worker was assumed to be white. This could artificially elevate the incidence of a particular cancer in the black population by dilution of the total population size. However, it is also possible that the excess in black workers could be due to job segregation by race, which may lead to higher average exposures for black workers. If the data are truly indicative of a causative association with chemical exposure at the plant, then based on the overall evidence, butadiene rather than styrene is more likely to be the agent involved.

In a more recent report of this cohort, measured exposure data were available for seven of the eight plants (Matanoski *et al*, 1993). The data were obtained by sampling exposures for particular jobs, and the jobs sampled varied between plants; thus, there may be differences in the measured levels for each plant dependent on the jobs sampled in each. These exposure data indicate that geometric mean exposure was higher in three of the seven plants (1.25-1.90 ppm) compared with the others and so the cohort mortality data were re-analysed for workers at these three plants only; this analysis was restricted to workers hired before 1960 and with 10 or more years of employment duration, a total of 3429 employees.

Mortality from all causes and all cancers was slightly reduced compared with expected values, with SMRs of 0.86 (95% CI: 0.80-0.92) and 0.96 (95% CI: 0.83-1.09) respectively. However, there was a statistically significantly elevated SMR for all LHS cancer (SMR = 1.63 95% CI: 1.13-2.27, 34 deaths), which was mainly attributable to a statistically significant excess of leukaemia and aleukaemia (SMR = 1.81; 95% CI: 1.01-2.99, 15 deaths). Although elevated SMRs were found for the other specific LHS cancer types, none of these reached statistical significance. Thus, increased SMRs of 1.16 (95% CI: 0.37-2.70, 5 deaths), 2.43 (95% CI: 0.78-5.68, 5 deaths) and 1.49 (95% CI: 0.68-2.82, 9 deaths) were found for lymphosarcoma and reticulosarcoma, Hodgkin's disease and cancers of other lymphatic tissue, respectively. This further analysis therefore supports the findings for the overall cohort and is suggestive of an excess of LHS cancer amongst longer-term workers who would be expected to have the highest exposures. However, given that the exposure data relate to general levels in each plant, and are not necessarily reliable indicators by which to compare exposures between plants, nor can they be related to individual exposure levels, they are of limited reliability.

A very large cohort mortality study conducted in workers in the US and Canada has very recently been completed (Delzell *et al*, 1995, 1996; Macaluso *et al*, 1996). This study combines the mortality experience of previously studied SBR worker cohorts, from workers at seven of the eight SBR plants in the US and Canada previously reported by Matanoski and Schwarz (1987) and Matanoski *et al* (1990, 1993) and from the US SBR two-plant complex previously reported by Meinhardt *et al* (1982). Only male workers who had worked at any plant for at least one year within the period of the study, 1943-1992, were included in the study cohort. In order to identify those eligible for inclusion, records were reviewed for 25,500 employees from seven US plants and for 6994 employees from the Canadian plant. Of the 25,500 US plant employees, a total of 12,605 were included in the study, and from the Canadian plant 5359 subjects met the eligibility criteria, of whom 3044 were identified as having worked in SBR and related operations. The remainder were classed as having worked in non-SBR related operations. The results presented below are for a total of 15,649 male workers who were known to have worked in SBR and related operations, a large number of whom are likely to have been included in the earlier epidemiological studies.

Payroll status (hourly-paid, salaried or mixed), year of hire, duration of employment, race and vital status was established for each cohort member. Cause of death was established from death certificates and classified according to ICD8. SMRs were calculated with reference to national US rates for the 7 US plants and to Ontario rates for the Canadian plant. An attempt was made to estimate exposures to butadiene, styrene and benzene, using work histories for about 97% of the study cohort. Analyses of process and job types were used in conjunction with an exposure model to estimate 8-hour TWAs and the number of exposure peaks, defined as an average exposure concentration of >100 ppm butadiene or >50 ppm styrene in any 15 minute period.

Vital status information indicated that as of January 1st 1992, of the 15,649 subjects in SBR and related operations, 10,939 (70%) were alive, 3976 (25%) were deceased and 734 (5%) were lost to follow-up. The average period of follow-up was 24.7 years per person, with a total of 286, 172 person-years of follow-up. The median year of hire was 1960 and 44% of the cohort had > 10 years employment since hire.

The SMR for death from all causes was 0.87 (95% CI: 0.85-0.90; 3976 deaths) and for all cancers was 0.93 (95% CI: 0.87-0.99; 950 deaths). The only cause of death for which an elevated SMR was found was for leukaemia, with an SMR of 1.31 (95% CI=0.970-1.74, 48 deaths). All other SMRs were close to or below 1.00. The cohort was subdivided according to duration of employment and number of years since hire. The subgroup with relatively long duration of employment (>10 years) and long period since hire (> 20 years) was found to have SMRs for all deaths and all cancers similar to that for the total cohort (all mortality SMR = 0.94, 95% CI = 0.90-0.99, 1678 deaths; all cancers SMR = 0.95, 95% CI = 0.86-1.04, 426 deaths). There was a statistically significantly elevated SMR for all lymphopoietic cancer in this subgroup (SMR = 1.39, 95% CI=1.04-1.83, 52 deaths). This was due mainly to an excess of mortality from leukemia, for which an SMR of 2.01 (95% CI=1.34-2.88, 29 deaths) was obtained. Also in this subgroup, elevated SMRs were recorded for laryngeal cancer (SMR = 1.41, 95% CI=0.65-2.68, 9 deaths) and cancer of the CNS (SMR=1.35, 95% CI=0.72-2.30, 13 deaths), although neither of these were statistically significant. All the deaths from laryngeal and CNS cancers were among white workers in this subgroup.

Further analysis indicated that the SMRs for 'ever-hourly' subjects, i.e. hourly-paid subjects, in jobs most likely to involve exposure to butadiene or styrene, were similar to those for the overall cohort, with again, a statistically significantly elevated SMR for leukaemia (SMR = 1.43, 95% CI=1.04-1.91, 54 deaths). In particular, 'ever hourly' workers with > 20 years since hire had an excess of leukaemia (SMR = 2.24, 95% CI=1.49-3.23, 28 deaths).The SMRs for never-hourly subjects were, in general, lower than those for ever-hourly workers, and the SMR for leukaemia was below 1.00 for this group. Among ever-hourly workers, the SMR for leukaemia increased with duration of employment (from 0.95 in subjects with < 10 years employment, to 1.70 in subjects with 10-19 years employment, and 2.40 in subjects with < 20 years of employment) and number of years since hire (SMRs of 0.50, 2.51 and 1.40 for < 20, 20-29 and $\geq$ 30 years since hire respectively). The leukaemia excess in ever-hourly workers was concentrated among workers hired in or after 1950, and with $\geq$ 10 years employment and 20-29 years since hire (SMR = 3,53, 95% CI=1.76-6.31, 11 deaths). No excess of leukaemia was observed in ever-hourly workers hired pre-1950; this group also had significantly low all-cause mortality. For all ever-hourly leukaemia decedents, median values of 58 years for age at death, 17.1 years employment duration, 28.2 years since hire and 1951 for year of hire were obtained.

When race was taken into consideration in the analyses, the leukaemia SMR was elevated for both white and black workers, and again, the excess was concentrated among ever-hourly workers with > 10 years employment and > 20 years since hire. The leukaemia excess in this group was higher in black workers (SMR=4.36, 95% CI=1.76-9.01, 7 deaths) compared with whites (SMR = 1.92, 95% CI=1.19-2.94, 21 deaths).

Work history data for 13,713 subjects were used to identify 5 main process groups – production (50% of subjects), maintenance (32%), labour (35%), laboratories (13%) and other operations (21%). These categories were not mutually exclusive. In general, although not always, SMRs were lowest for laboratory workers and highest for maintenance workers. Statistically significant excesses were found for lung cancer among maintenance workers (SMR=1.24, 95% CI=1.04-1.46, 141 deaths) and for leukaemia among production workers (SMR=1.59, 95% CI=1.00-2.41, 22 deaths), labourers (SMR=1.95, 95% CI=1.12-3.17, 16 deaths, concentrated among black workers), laboratory workers (SMR=4.62, CI=2.38-8.06, 12 deaths, all white workers) and among black workers in other operations (SMR=6.80, 95% CI=1.37-19.86, 3 deaths). The two job categories with the highest SMRs for leukaemia, laboratory workers and maintenance labourers, involved intermittent exposures to high levels of butadiene and styrene.

Estimated exposure to butadiene monomer, styrene and benzene were based on data from six of the eight plants. Cumulative mean 8-hour TWAs estimated for all exposed workers were 11.2 ppm-years for butadiene, 7.4 ppm-years for styrene and 2.9 ppm-years for benzene. Leukaemia decedents had higher mean cumulative exposures (36.4 ppm-years

butadiene, 22.4 ppm-years styrene and 5.5 ppm-years benzene) in comparison with all other exposed workers or with all decedents. Benzene exposure was low and infrequent and showed no association with leukaemia and was therefore excluded as a potential confounding factor. Regression analysis was performed to evaluate the association between cumulative exposure to butadiene and styrene separately. A positive association was found between cumulative exposure to butadiene and leukaemia mortality, after controlling for styrene exposure, age, years since hire, calendar period and race. The association between cumulative styrene exposure and leukaemia, corrected for butadiene exposure and the co-variables listed above did not show a consistent trend with exposure. RRs of 1.0, 1.0, 1.2, 1.8 and 1.3 were calculated for cumulative exposures to styrene of 0, >0-19, 20-39, 40-59 and $>$ 60 ppm-years, respectively.

However, more recently, following concerns raised about the validity and accuracy of the exposure estimates used in this analysis, a re-evaluation of the exposure estimates was performed, taking into account new information that allowed a refinement of the original estimates (Macaluso et al, 2000, unpublished report and Delzell *et al*, 2001). The revised assessment found that the exposure estimates originally derived were likely to have underestimated exposures to butadiene and styrene. Based on these revised exposure estimates, the relationship between exposure to butadiene, styrene, and another recently identified potential confounder, dimethyldithiocarbamate (DMDTC) and LHS cancers was (re-)investigated.

The analysis using the revised exposure estimates included 13,130 of the original 17,964 eligible workers at the eight SBR plants. As before, inadequate information was available for two of the plants, to allow a reliable estimate of exposure to be derived and therefore all eligible workers (1354) from these two plants were excluded. Additional exclusions were made on the basis of duplicate records (12) or because subjects had died or were lost to follow-up before reaching 40 years old or more than 10 years since hire. The re-evaluation did not include exposure to benzene, as exposures were low and there was no association with LHS cancer in previous analyses. The analysis investigated the association with all forms of leukaemia and with specific sub-types. Relative rates (RRs), determined by regression analysis, were calculated for exposed workers compared with unexposed or low exposed workers, based on exposure level, age and years since hire. Vital status was determined for $\geq$ 99% of the cohort and information from death certificates and from medical records where available, was used to confirm cause of death from LHS cancer. Of all LHS cancer deaths, 59 were leukaemia, 38 were non-Hodgkin's lymphoma (NHL), 21 were multiple myeloma and 9 were Hodgkin's disease.

The median cumulative exposures were 17 ppm-years for styrene and 71 ppm-years for butadiene; exposure to DMDTC was by the dermal route only, and cumulative exposure was estimated to be 374 mg-years.cm^{-2}. For all decedents, median cumulative exposures were 18 ppm-years for styrene, 90 ppm-years for butadiene and 836 mg-years.cm^{-2} for DMDTC. Subjects with leukaemia and NHL had higher median exposures to styrene, butadiene and DMDTC and subjects with multiple myeloma had higher styrene and butadiene exposures compared with all decedents.

Regression analysis indicated a positive association between cumulative exposure to butadiene and leukaemia. RRs (adjusted for age and years since hire) were 1.0, 1.2, 2.0 and 2.8 for butadiene exposures of 0, >0-<86.3, 86.3-<362.2 and $\geq$ 362.2 ppm-years, respectively. A positive association with leukaemia was also found for cumulative exposure to styrene, with adjusted RRs of 1.0, 1.2, 2.3 and 3.2 for exposures to 0, >0-<20.6, 20.6-<60.4 and >60.4 ppm-years, respectively. The RRs reached statistical significance only for the highest exposure category, in each case. Similarly, after controlling for age and years since hire, the total number of butadiene peaks (any exposure >100 ppm) and styrene peaks (any exposure >50 ppm) was positively associated with leukaemia. However, after adjusting the RRs for exposure to both butadiene and DMDTC, there was no positive association

between cumulative styrene exposure or with exposure to styrene peaks and leukaemia. When adjustment was made for butadiene exposure alone, a positive relationship between cumulative styrene exposure and leukaemia remained, although the RRs were reduced and none reached statistical significance. There were no notable associations for any other LHS cancer sub-groups, although the small numbers precluded detailed analysis.

Overall, this large cohort-mortality study shows a clear excess of leukaemia among workers in the SBR industry. The excess is concentrated among hourly-paid workers, in jobs with the potential for the highest exposures, and with long durations of employment (> 10 years) and long time since hire (> 20 years). However, the evidence does not indicate any causal association with occupational exposure to styrene. There is no consistent trend in mortality rates with increasing styrene exposures, particularly when correction is made for the other occupational exposures in these plants.

In a mortality study at one tyre-manufacturing plant in Akron, Olio, at which SBR manufacture was one of a range of processes, all 6678 male workers and retirees aged 40 or over on 1 January 1964 were entered into the study (McMichael *et al*, 1974, 1975, 1976). A total of 1783 deaths were recorded, all those occurring between 1964 and 1973; death certificates for all but nine were obtained, classified in accordance with ICD8 and compared with age-specific US national mortality data.

The number of deaths from all causes was similar to that expected (SMR = 0.99). However, among specific cancer types, there was a statistically significant excess mortality due to lymphosarcoma (SMR = 2.26, 95% CI: 1.24-3.79, 14 deaths), cancer of the stomach (SMR=1.87, 95% CI: 1.33-2.56, 39 deaths) and cancer of the prostate (SMR = 1.42, 95% CI: 1.05-1.88, 49 deaths). Deaths among those aged 40-64 were also analysed separately. The number of deaths from all causes was similar to that expected (SMR = 0.93), but there was statistically significantly elevated mortality from leukaemia (SMR = 3.15, 95% CI: 1.57-5.64, 11 deaths) and cancer of the stomach (SMR = 2.19, 95% CI: 1.13-3.82, 12 deaths), with an excess of lymphosarcoma seen, although this did not reach statistical significance (SMR = 2.51, 95% CI: 0.92-5.46, 6 deaths). Further analysis showed that an employment record including at least 5 years in the synthetic plant (where predominantly butadiene-styrene rubber was manufactured) was 5.6 times as common among decedents from LHS cancer than in a representative sample of about one quarter of the workforce.

In the context of other studies, this study appears to show an excess of LHS cancers among workers, with evidence of an association between leukaemia and lymphosarcoma and exposure to butadiene in an older subgroup of the population.

Following on from earlier work by Siemiatycki *et al*, 1981, in a population-based case-control study in Montreal, Canada, the work history of 142 males with confirmed renal cell carcinoma during the period 1979-1985 (out of a possible 277 cases) was detailed by questionnaire (Parent *et al*, 2000). A population control group of 533 workers and 1900 people with cancer at any other site was identified. The questionnaire addressed a number of lifestyle risk factors for cancer such as smoking and body-mass index, and a detailed work history from which a retrospective, qualitative, exposure assessment was constructed. Exposure was graded as possible (<5% of the working week), probable (5-30% of the working week), or definite (>30% of the working week). Also the intensity of the exposure was graded as low, medium or high.

A total of 10 cases were attributed to the styrene-butadiene rubber industry giving an odds-ratio of 1.8 (95% CI = 0.9-3.7) when adjusted for other possible occupational confounding factors. The authors remarked that this was of ‘borderline statistical significance’. It is difficult to draw firm conclusions from such a population-based study, as there are uncertainties about job and exposure classification, also because of problems in fully addressing potential confounding factors. Furthermore, although an association is drawn with a type of industry, this study has not achieved a clear identification of the causative agent.

Similarly, the same team evaluated possible risk factors for rectal cancer using a population-based case control design (Dumas *et al*, 2000). Out of a possible 304 cases 257 were interviewed. A population control group of 533 workers and 1295 people with cancer at any other site (excluding those with lung cancer, and other intestinal sites) was identified.

There were 6 cases of rectal cancer associated with 'any' exposure to styrene, giving an odds ratio (OR), adjusted for a number of lifestyle factors but not other potential occupational confounders, of 1.7 (95% CI = 0.7-4.5) and 5 cases associated with 'substantial' styrene exposure, with an OR of 3.9 (CI = 1.2-12.9). As with the previous study, there is still the potential for exposure misclassification. Statistical power is low because of the small number of subjects and the low exposures generally encountered. This study analysed nine other solvents, all of which showed an increased risk of rectal cancer and it is likely that there has been mutual confounding. Overall, as with the previous study, no firm conclusions can be drawn.

The cohort from eight SBR facilities reported in a mortality study in the previous part of this section (Matanoski et al, 1990) was investigated further in a nested case-control study (Santos-Burgoa et al, 1992). This study considered specifically the association between exposure to butadiene and/or styrene and the incidence of LHS cancer. A total of 59 LHS cases were identified from the cohort, with cause of death from LHS cancer confirmed from death certificates. A total of 193 controls were selected from workers in the cohort who were alive or who had died from causes other than cancer and who had survived at least as long as the case. Controls and cases were matched for age, year of first employment, duration of employment and plant. Job history was defined for each subject and an exposure estimate was made by a panel of 4 chemical engineers with practical experience of the industry, and by an environmental engineer. This panel ranked jobs according to the extent of exposure to butadiene, styrene and to other potentially toxic chemicals. However, as exposure to other chemicals was a rare event, these exposures were considered negligible. Ranked exposures to styrene and butadiene were given a score of 1-10 with 10 representing the highest exposure, and an exposure index was calculated as the product of the exposure score for each job held and number of months spent in that job. Each matched case-control set was classified to two exposure classes according to whether the case exposure score was greater or less than the geometric mean of the exposure scores of the controls. Odds ratios (OR) were calculated for all LHS cancers and for four lymphohematopoietic cancer subgroups.

Of the 59 LHS cancer cases, there were 6 lymphosarcomas, 8 Hodgkin's disease, 26 leukaemias and 18 other lymphatic neoplasms. On the basis of the exposure indices, cases of leukaemia had considerably higher scores for butadiene and styrene exposure than did control, or the other cancer subgroups. Odds ratios were calculated for workers ever/never exposed to butadiene and styrene, in an unmatched analysis. Only in the case of leukaemia was there a significant increase in the OR for both butadiene (6.82, 95% CI: 1.10-42.2) and styrene and (4.25, 95% CI: 1.02-17.8). Further analysis of the matched pairs using the dichotomised exposure scores resulted in an increase in the OR for butadiene (OR = 9.36, 95% CI: 2.05-22.9), whereas the OR for styrene was reduced by this method and was no longer statistically significant. The only other association that reached statistical significance was between butadiene exposure and risk of all LHS cancers (OR = 2.3, 95% CI: 1.13-4.71).

Conditional logistic regression models were used to calculate the odds ratios for exposure to butadiene adjusted for styrene exposure as a confounding variable. Separate exposures to either butadiene or styrene occurred infrequently, so that the reliability of such an analysis is reduced. However, for all LHS cancers, the odds ratio for butadiene exposure alone was 2.42 (95% CI: 1.12-5.23). A very strong association was found between exposure to butadiene alone and cases of leukaemia (OR = 7.61, 95% CI: 1.62-35.6, $p<0.002$). In comparison, the OR for styrene exposure alone was not statistically significantly increased; OR 2.92 non-significant. Including both exposures as explanatory variables gave an

adjusted OR of 7.39 (95% CI 1.32-41.3) for butadiene but that for styrene was reduced further to 1.06 (non-significant).

Overall, this study demonstrates a strong association between exposure to butadiene and incidence of LHS cancer, particularly leukaemia. This association is increased when the potential effect of co-exposure to styrene is taken into account and therefore strongly implicates butadiene as the chemical of concern with respect to these data. Exposure to styrene alone was not associated with any significant excess cancer risk.

A re-analysis of these data was conducted in order to address the possibility that effects in the most highly exposed workers were underestimated because of inclusion of large numbers of unexposed workers in the overall cohort (Matanoski *et al*, 1993). It was noted that for most areas of the plant, the range of exposures included zero exposure, so that effects on workers with high exposures could potentially be diluted by inclusion of workers with little or no exposure. In addition, measured butadiene exposure data from some of the plants were compared with the rank exposure scores used in the model and a reasonable correlation was found. However, when measured styrene exposure data were compared with the ranking used in the previous study, no significant correlation was found; therefore nothing can be concluded about exposure to styrene from these studies. The same cohort was used in this analysis, and again, condition logistic regression models were used to calculate odds radios for butadiene exposure adjusted for different confounding variables. It was found that there was an excess risk for leukaemia associated with 3 particular work areas, operation services, laboratory and utility. In these 3 areas, jointly referred to as mixed jobs, the OR for leukaemia was 3.8 (95% CI: 1.2-11.9). Overall, these data support the findings of the original case-control study with regard to carcinogenicity of butadiene but no conclusions can be drawn about any causal association with exposure to styrene.

In a follow-up to the above Matanoski study, the original authors have re-analysed the cancer incidence data to determine the relative risk of styrene exposure and an association with lymphohaematopoietic cancers using nested case-control analyses (Matanoski *et al*, 1997). Exposure measurements were collected from 7/8 plants via personal monitoring and exposure data obtained from NIOSH (data not reported). Cases of cancer were confirmed through a hospital record review of 95% of the reported cancers. The calculated odds ratios for an association with low level styrene exposure at 1 ppm suggest an apparent association between the continuous measure of styrene exposure and all LHS cancers – OR 2.2 (95% CI, 1.460-3.33); lymphoma OR 2.6 (95% CI, 0.40-17.15); all lymphomas OR 2.7 (95% CI, 1.22-5.84); lymphosarcoma OR 3.9 (95% CI, 1.6-9.6) and multiple myeloma OR 3.0 (95% CI, 1.3-7.0). It should be noted that the workers may also have been exposed to levels of butadiene, and therefore that a conclusive causal relationship between styrene exposure and cancer risk cannot be drawn from this study.

No significant excess of mortality from lymphatic and haematopoietic cancer was reported among students attending schools adjacent to styrene-butadiene facilities (Loughlin et al, 1999). A cohort of 15,403 students attending schools between 1963/63 and 1992/93 in Texas, which were located next to facilities producing synthetic styrene-butadiene was investigated. No information regarding possible styrene or butadiene exposure levels in the schools was reported. Amongst the cohort 338 deaths were identified, of which 14 (12 in males and 2 in females) were reportedly due to LHS cancers. These rates were compared with nationally expected rates in the US, giving an SMR for all cases of 1.21 (95% CI 0.66-2.04). This study provides no evidence of any increased incidence of mortality from LHS cancers in these students.

Ecological studies

In an ecological study aimed at investigating the role of the environment in breast cancer development, an association was found for styrene between its industrial release/emission levels during 1988-2000 and the average annual age-adjusted breast cancer rates for the 1995-2000 period among different counties of Texas (Coyle et al., 2005). It is considered that no toxicological significance should be attributed to this statistical association because of a number of limitations identified in the design and conduct of the study. First of all, there is no information in the study on the actual magnitude or length of exposure to styrene that the individual breast cancer cases may have experienced before they took up residence in the various Texas counties where they were diagnosed with breast cancer, as well as how long they lived in these counties. Secondly, although the study took account of age and ethnicity, no attempt was made to control for several important breast cancer risk factors which are normally found associated with more industrialised areas (nulliparity, age at first child birth, use of contraceptives, obesity, etc..). Furthermore, it should be noted that this positive association, which was found for very low levels of environmental exposure to styrene, stands in contrast to the negative findings of several studies conducted in occupational settings where exposures to styrene are a lot higher.

Summary of studies in humans

In summary, several cohort and case-control studies covering workers exposed to styrene are available. In large, well-conducted studies, cancer mortality was investigated in the GRP industry with relatively high exposure to styrene and no significant exposures to other chemicals. In these studies, there was no clear evidence for a causal link between specific cancer mortality and exposure to styrene. In the styrene-butadiene rubber industry, several studies have pointed to an increased risk of cancer of the lymphatic and haematopoietic systems. However, detailed analysis of these data, together with the general toxicological picture for styrene and butadiene (see butadiene EU RAR), suggests that where increases are due to occupational exposure, it is butadiene, not styrene, that is the causative agent. In conclusion, based on human studies, there is no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene.

4.1.2.8.3 Summary of carcinogenicity

In relation to human studies, several cohort and case-control studies covering workers exposed to styrene are available. In large, well-conducted studies, cancer mortality was investigated in the GRP industry with relatively high exposure to styrene and no significant exposures to other chemicals. In these studies, and in studies in styrene production workers, there was no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene. The increased risks for lymphatic and haematopoietic neoplasms observed in some of these studies are generally small, statistically unstable and often based on subgroup analyses. These findings are not very robust and the possibility that the observations are the results of chance, bias or confounding by other occupational exposures cannot be ruled out. In the styrene-butadiene rubber industry, several studies have pointed to an increased risk of cancer of the lymphatic and haematopoietic systems. However, detailed analysis of these data, together with the general toxicological picture for styrene and butadiene (see butadiene EU RAR), suggests that where increases are due to occupational exposure, it is butadiene, not styrene, that is the more likely causative agent. In conclusion, based on human studies, there is no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene.

The carcinogenic potential of styrene has been explored in rats and mice, using the inhalation and oral routes of exposure. A carcinogenic effect of styrene towards the lung is

evident in the mouse. This has been shown in a well-conducted lifetime inhalation study in CD1 mice at exposure concentrations of ≥ 20 ppm styrene and, somewhat less convincingly, in an oral study in mice of the $B6C3F_1$ strain. The inhalation study, which included extensive histopathological examination, showed that the tumours (prevalently adenomas) were preceded by cytotoxicity characterised by early Clara cell toxicity followed by progressive bronchiolar epithelial hyperplasia and bronchiolar-alveolar hyperplasia.

In the rat, styrene has not exhibited any clear evidence of carcinogenic potential by the inhalation or oral route. In individual studies there have been isolated findings of statistically significantly higher incidences of various particular tumour types in particular groups of styrene-treated animals, compared with the in-study controls. However, the findings have been within historical background ranges, not reproducible between studies, in some cases have not shown an upward trend with increasing dose, and have not been associated with evidence of underlying styrene-induced changes at the site in question.

On the question of the relevance of the mouse lung tumours for human health, consideration of the available toxicokinetic information and data from single and repeated inhalation exposure studies in experimental rodents suggests the following as the most plausible toxicological mechanism for the mouse lung tumours. Styrene is metabolised by cytochrome P450 enzymes in the metabolically active Clara cells (non-ciliated bronchiolar epithelial cells involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium) of the bronchiolar epithelium of the mouse, producing cytotoxic metabolites of styrene including styrene 7,8 oxide (SO) and oxidative metabolites of 4-vinylphenol (4-VP). These metabolites cause early Clara cell toxicity/death and sustained regenerative bronchiolar cell proliferation which, in turn, leads to compensatory bronchiolar epithelial hyperplasia and ultimately tumour formation. Clara cell toxicity could also be a consequence of the long term depletion of glutathione, because of conjugation with SO. Genotoxicity of SO (an EU-category 2 and IARC group 2A carcinogen) or other reactive styrene metabolites is unlikely to be involved in tumour development as minimal binding of styrene metabolites to DNA has been detected in mouse lung with no species- or tissue-specificity.

All of the key events of this postulated mode of action are less operative in the non-responsive rat (which does not develop lung tumours at exposure concentrations up to 1000 ppm) and even less operative in humans.

The number of Clara cells (being responsible for both the formation of toxic metabolites and the target for their toxic action) is very low in humans, even less than in rats. While Clara cells comprise about 85% of bronchiolar epithelium in mice and 25% in rats, in humans such cells are rare.

Although the enzymes CYP2E1 and CYP2F2 required for the formation of the Clara cell toxicants such as SO (including the highly pneumotoxic R-enantiomer) and the downstream metabolites of 4-VP have been detected in human lung, their activities are low (at least 400 times lower than in the mouse) and metabolic activation of styrene to SO is minimal or undetectable.

In human lung, detoxification of SO (if formed at all in human pulmonary tissue) takes place predominantly *via* epoxide hydrolase (located on the endoplasmatic reticulum in close proximity to the toxifying cytochrome P450s). The close proximity of the “detoxifying” enzymes to any “toxifying” enzymes ensures the efficient removal of any toxic metabolites. Rodents use both epoxide hydrolase and glutathione-S-transferase as detoxification pathways with the mouse relying on glutathione conjugation more so than the rat. As glutathione S-transferase is located in the cytosol, this makes this detoxification pathway less efficient than the epoxide hydrolase pathway. In comparison to the rodent species, in humans, SO detoxification proceeds nearly exclusively via epoxide hydrolase and glutathione S-transferase accounts for only 0.1% of SO detoxification.

Taking account both of the toxification to SO and its detoxification, PBPK-modelling has shown that the SO content of human lungs is very small, if there is any.

Formation of 4-VP and its downstream metabolites occurs at a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse concentrations). Although it cannot be ascertained whether or not these species differences in the formation of 4-VP metabolites in the lung may be a reflection of the different numbers of Clara cells (the metabolically active lung cells) present in the different species, since 4-VP metabolites are produced by the same cytochrome P450 enzymes involved in the production of SO, it is most likely that the species differences in the formation of 4-VP metabolites observed reflect species differences in metabolic capability.

As indicated by PBPK-modelling, glutathione depletion caused by SO does not occur in humans. Also, as reactive downstream metabolites of 4-VP are formed in human lung only to a very small extent, the 4-VP metabolic pathway is not expected to cause any glutathione depletion in human pulmonary tissue.

There is no evidence from extensive epidemiological investigations that long term exposure to styrene has produced lung damage or lung cancer in humans.

Hence, overall, the weight of evidence appears to indicate that the consequences of long term exposure to styrene in mouse lung cannot be replicated in the human situation at relevant levels of exposure. Although there are still some uncertainties in this postulated mode of action and in its relevance to humans, namely the lack of data on the relative rates of 4-VP metabolites detoxification in different species, no alternative modes of action that logically present themselves can be supported by as significant a body of evidence as the one presented in this assessment. Consequently, it is felt that the level of confidence in the postulated mode of action can be reasonably high and that, in view of the extensive negative lung epidemiology, it is reasonable to conclude that the lung tumours seen in mice are unlikely to be of any relevance for human health. A more detailed analysis (according to the IPCS framework for evaluating a mode of action in chemical carcinogenesis) of the evidence in support of the proposed mode of action and of its relevance for human health is presented in Annex A to this document.

The carcinogenicity of styrene was evaluated by IARC in 2002. Styrene was considered *possibly carcinogenic to humans* (Group 2B). The Working Group concluded that based on metabolic considerations, it is likely that the proposed mechanism involving metabolism of styrene to styrene 7,8-oxide in mouse Clara cells is not operative in human lungs to a biologically significant extent. However, based on the observations in human workers regarding blood styrene 7,8-oxide, DNA adducts and chromosomal damage, it cannot be excluded that this and other mechanisms are important for other organs.

In the Rapporteur's view, pointing to a possible carcinogenic potential of styrene in other organs is highly speculative as: a) Several large cohort and case-control studies of workers exposed to styrene have shown no evidence for a causative association between styrene exposure and cancer in humans at any site; b) No consistent evidence for styrene-induced toxicity in any organ has emerged from studies of exposed workers; c) The level of DNA damage found in workers exposed to styrene is very low (10-fold lower than that produced by endogenously-generated genotoxic substances such as ethylene oxide) and thus cannot be considered to be of any relevance for subsequent tumour formation. Mechanistic studies have shown that styrene-oxide (SO) and its genotoxicity are not the driving force for lung tumour formation in mice, the only experimental tumour site observed so far. Furthermore, DNA adducts in animals after styrene exposure do not show any specific species or target organ relationship. For example, there is no excess of SO-adduct formation in tissues where SO is formed (e.g. in the liver) at high levels; d) Chromosomal damage caused by styrene exposure in humans is far away from being conclusive. Although 5 studies appear to present evidence that styrene may be weakly clastogenic in humans, there are 11 robust negative

studies also. Together with a lack of evidence of a dose-response relationship and the negative response for induction of micronuclei when studied concurrently in two of the positive chromosome aberration studies, no clear conclusion on *in vivo* clastogenicity of styrene in humans can be made. Furthermore, at much higher exposures such effects were not observed in experimental animals.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies of endocrine disruption activity

In vitro

Oestrogenic activity

An "E-screen" assay conducted by Soto *et al* (1995) has been reported. Human MCF-7 breast cells (which have the oestrogen receptor) showed no evidence of increased cell proliferation (an indicator of oestrogenic activity) when treated with styrene. Negative results were also reported in another study investigating the ability of styrene monomer, dimers and trimers to induce proliferation in human MCF-7 breast cells (Nobuhara *et al*, 1999). The same authors also reported negative results in an oestrogen binding assay (no binding to oestrogen receptor) with styrene monomer and oligomers. In a review prepared for the Styrene Producers Association (1999), negative results are also reported in an oestrogen binding study using extracts from polystyrene in an oestrogen mediated recombinant receptor gene assay (Fail *et al*, 1998).

As part of a study to test the potential endocrine-disrupting effects of styrene oligmers, the styrene monomer was tested in three *in vitro* assays (Ohno *et al,* 2001*)*. In an œstrogen receptor binding assay styrene failed to demonstrate a significant inhibitory effect against binding of [^{3}H]œstradiol to the receptor. In a luciferase assay styrene failed to induce any significant luciferase activity, and in a MCF7 cell proliferation assay styrene failed to elicit any significant cell growth. In all three assays the positive controls 17β-œstradiol and diethyl-stilbœstrol gave the appropriate response. Overall, these results demonstrate that under the conditions of this study styrene has no œstrogenic activity.

Androgenic activity

No evidence of any ability for receptor binding or inhibition of testosterone production was observed in an investigation of the androgenic and anti-androgenic activity of styrene monomer, dimers and trimers in rat Leydig cells (Nobuhara *et al*, 1999).

In vivo

Uterotrophic assays

An uterotrophic assay in rats examining styrene monomer, dimers and trimers is available (Nobuhara et al, 1999). Groups of female rats were administered doses of up to 200 mg/kg subcutaneously once a day for 3 consecutive days. Twenty-four hours after the last dose, the uteri and vaginas of the animals were excised, the uteri weighed and both tissues examined histopathologically. No evidence of any treatment-related effects on uterine weights or tissue histopathology was observed.

In a review prepared for the Styrene Producers Association 1999, negative results are also reported from uterotrophic assays in rats following “high” oral doses of extracts of polystyrene (Fail *et al*, 1998; Bachman *et al*, 1998; Klaerner *et al*, 1998).

Prolactin studies

Eighteen male Sprague-Dawley rats were given a single intra-venous injection of 5.8 mg styrene in aqueous Tween over 1 hour. Nine controls received vehicle only. The dose was selected using a PBPK model to equate to an inhalation dose of 300 ppm. Blood was collected via an in-dwelling jugular vein catheter at intervals of 15 minutes, 4 times prior to dosing, 4 times during dosing and 8 times post-dosing. Measurements of prolactin (PRL), GH, LH and corticosterone were conducted using radio-immunoassays. Individual animal body weights were measured during the study duration and were comparable with controls. No treatment-related changes in the blood serum levels of PRL, GH, LH or corticosterone, compared with controls, were observed (Wuttke *et al*, 2000).

Male rats were exposed to styrene by inhalation (0, 645, 2150 and 6450 mg/m^3 equivalent to 0, 148, 495 and 1484 ppm) for 6h/day on 5 consecutive days. Serum dopamine and PRL levels as well as concentrations of catecholamines and their metabolites in the striatum and mediobasal hypothalamus were determined either directly after the last exposure or after a recovery period of 24 h. There was no statistically significant change in any of these parameters (Jarry et al., 2002).

Groups of 8 male and 8 female Wistar rats, 8 weeks of age, were exposed by whole body inhalation to 0 or 150 ppm (650 mg/m^3) styrene vapour 8h/day for 10 days (Umemura et al., 2005). Following exposure, the animals were sacrificed and blood and brain samples collected. Styrene concentration in blood, hormones such as PRL, growth hormone (GH) and TSH in plasma and neurotransmitters (dopamine, 5-hydroxytryptamine and their metabolites) in various brain regions were measured. Styrene concentration in the blood of female rats was higher than in male rats (43.4 ng/ml vs 21.4 ng/ml). Plasma PRL levels were significantly increased in female exposed rats compared to controls (32.2 ng/ml vs 24.2 ng/ml). No significant change was observed in male rats. There were no significant changes in the brain levels of dopamine, 5-hydroxytryptamine or their metabolites. These results seem to suggest that the sensitivity to styrene exposure of female rats may be higher than that of the males and that styrene exposure to 150 ppm for 10 days produces increased levels of plasma PRL in female rats. However, in the absence of information on the normal background levels of PRL in the rat, this relatively minor increase in plasma PRL which was investigated only at one exposure concentration and at one time-point is unlikely to be of toxicological significance. It should also be noted that this result is inconsistent with the observations of the previous study in which no effects were seen for exposures up to approximately 1500 ppm styrene.

Other studies

Reduced levels of testosterone were observed in male mice exposed to 12 mg/kg/day styrene for 4 weeks by Takao et al (2000), but the full toxicological significance of this finding is unclear given the absence of histopathological findings in the testes. Interpretation is also made difficult by the limited range of examinations (observations were made at one single point), the absence of background information on the possible normal range of testosterone levels that might be expected in these young mice and the absence of such effects at such low dose levels in other well documented studies. See section 4.1.2.6.1 for further details of the study.

Styrene-induced oestrous cycle changes in mice have been reported in a repeat inhalation study (abstract only available). See section 4.1.2.6.1 for further details of the study methodology (Roycroft *et al*, 1992).

Three poorly reported studies are available (Bakhtizina et al, 1982; Bakhtizina et al, 1983; Bakhtizina and Popuchiev, 1981), which apparently suggest that inhalatory exposure to styrene at an unspecified 'limiting' concentration for an undetermined period had an effect on rat ovarian morphology and enzymic activity, and that subcutaneous dosing with 200 mg/kg/day for an unspecified time period resulted in compensatory ovarian hypertrophy after hemiovariectomy. Given the limited reporting of the methodology of these studies, no conclusions can be drawn from them.

Oestrous cycle was investigated in three poorly reported studies in the rat (Bondarevskaya, 1957; Izyumova, 1972; Izyumova *et al*, 1972; Zlobina *et al*, 1975). General toxicity including reduction in bodyweight was observed. The design of the experiments was not clear and therefore the results are not useful.

Overall, there is no evidence that styrene possesses significant endocrine disruption activity.

4.1.2.9.2 Reproductive toxicity studies in animals

Fertility studies

In an OECD- and GLP-compliant two-generation reproduction toxicity study, the effects of styrene on reproductive performance and fertility were evaluated (Unpublished, Stomp et al., 2003; Cruzan et al., 2005a). Included in the study was an assessment of the potential developmental neurotoxicity of styrene in the F_2 generation (Cruzan et al., 2005b). 25/sex/group Sprague-Dawley rats were exposed for 6 hours daily to either clean air or styrene vapour in a stainless steel and glass whole-body inhalation chamber. Styrene concentrations were 50, 150 and 500 ppm (216.5, 649.5, and 2165mg/m^3).

The F_0 generation was exposed for 10 weeks prior to mating and throughout the subsequent two weeks of mating, during which males and females from each group were randomly paired and co-habited. The females continued inhalation exposure during gestation and lactation, except from gestation day 21 through to lactation day 4, when styrene was administered in olive oil by gavage at dose levels of 66, 120 and 300 mg/kg/day (divided into 3 equal doses approx. 2 hours apart). This was done because this period is critical to pup neurological and neuroendocrine development and hence, there were concerns that stress on the pups arising from the removal of the dams for the 6h exposure session might have affected pup development. These oral dose levels were chosen (based on the Sarangapani physiologically based pharmacokinetic (PBPK) modelling) to generate peak blood levels of styrene after each gavage dose that closely matched the predicted blood level of styrene from each of the 3 inhalation exposure levels. F_0 males and females were sacrificed after the mating period and weaning of the F_1 pups respectively. All F_0 animals that died and those euthanized *in extremis* or at termination were subject to detailed macroscopic examination, and specific tissues and organs (reproductive tissues, lung, liver, adrenal, thyroid, spleen and kidney) were collected for histopathology. For all females that delivered, the uterus was examined to determine the number of implantation sites. For those that failed to deliver, the uterus was examined grossly for evidence of implantation.

At weaning, offspring (25/sex/group) were randomly selected to constitute the F_1 generation. Inhalation exposure of the F_1 animals was initiated on post-natal day (PND) 22 and, followed exactly the same protocol as for the F_0 generation. The non-selected offspring of both the F_0 and F_1 were sacrificed and subject to necropsy with specific emphasis on developmental

morphology and reproductive organs. The F_2 generation was not directly exposed to the test article but was potentially exposed *in utero* and through nursing during PND 0-21. At weaning, 40 F_2 pups/sex/group were selected for post-weaning developmental landmarks and neurobehavioural evaluation (i.e. functional observatory battery evaluations, locomotor activity, acoustic startle response and learning and memory evaluations). No exposure to styrene occurred during this period. In addition, 10 F_2 pups/sex/group were selected for neuropathological assessment (performed on PND 21), which included brain weight and brain dimension measurements, brain morphometric analysis and central and peripheral nerve evaluation. These data are described under the neurobehavioural/ neuropathological section of this summary.

Body weights, food consumption, food efficiency and detailed clinical examinations were regularly recorded throughout the study. Daily vaginal smears were performed for determination of estrous cycles, beginning 21 days prior to mating. Pregnancy outcome was monitored in both F_0 and F_1 generations at all doses, and terminal studies on both generations included comprehensive assessment of sperm measures (motility, caudal epididymal sperm counts and morphology). Ovarian primordial follicle and corpora lutea counts were conducted for all F_1 females in the control and high-exposure groups and for F_1 females in the mid-exposure group that did not mate or produce offspring. Developmental landmarks (pinnal detachment, surface righting response, hair growth, incisor eruption, eye opening, preputial separation and vaginal perforation) were assessed in all the selected F_1 and F_2 rats. Detailed microscopic evaluations of the reproductive tissues were performed for all F_0 and F_1 parental animals in the control and high-exposure groups and for all adult animals in the low- and mid- exposure group that were found dead or euthanized *in extremis*.

The principal finding of the study in the parental animals of the F_0 and F_1 generations was the degeneration of the olfactory epithelium lining of the nasal cavity observed in the high-exposure group only. This effect has been previously reported in rats following repeated inhalation exposure (see section 4.1.2.6.1). The degeneration was characterized by disorganisation and one or more of the following features: regenerative hyperplasia, individual cell necrosis and atrophy. There was also an increased presence of Bowman's glandular elements and cysts in the olfactory epithelium. The incidence and degree of degeneration were less pronounced in the F_1 generation compared to the F_0 generation. No evidence of this lesion was found at the lower concentration levels in either the F_0 or F_1 generations. In the high-exposure group of both the F_0 and F_1 generations, the mean body weight of the males was statistically significantly reduced by 7-8% (F_0) and 8-13% (F_1) compared to controls and in females by 7-8%. Mean body weights of the high-exposure females during gestation were reduced by 5% (not statistically significant) in the F_0 generation and by 6-7% (statistically significant) in the F_1 generation, indicating that there were statistically significant maternal effects on body weight only in the high-exposure females of the F_1 generation. In the mid-exposure group of the the F_0 and F_1 generations the mean body weight of the males was statistically significantly reduced by 6-7%. There were no statistically significant effects on body weights in the mid-exposure females of the F_0 and F_1 generations, although reductions of up to 5-6% were observed in the F_1 females during the study including gestation. Overall, there were no statistically significant maternal effects on body weight at 150 ppm. Water consumption measured in females during the gestation and lactation periods only was statistically significantly increased during gestation in the mid- and high-exposure groups of the F_1 generation (by 11-14% and 20-24% respectively) and in the high-exposure group of the F_0 generation (by 13-24%). Relative (to final body weight) liver weights were statistically significantly increased compared to controls in the 150 ppm and 500 ppm F_0 males by 6% and 15% respectively and in the 500 ppm F_1 males by 11.6% but the absolute liver weights were similar to controls. Also, statistically significant increases in relative weights of the brain were observed in the high-exposure males of the F_0 generation (by 7.4% of the control value) and in the mid- and high-exposure males of the F_1 generation (by 9.6% and 6.4% respectively compared to controls), but again the absolute

weights were similar to controls. The relative weight of the kidneys was statistically significantly increased in the mid- and high-exposure males of the F_0 generation by 5.8% and 10% respectively, but the absolute weights were similar to controls. All these increases in relative organ weights can be attributed to the decrease in mean body weights observed in these groups of animals, and hence, are considered of no toxicological significance.

Reproductive performance (i.e. mating behaviour and fertility), gestation length, litter data (number of pups, sex ratio), postnatal survival, sperm evaluations and primordial follicle counts were not adversely affected by styrene exposure across the generations. The mean length of the estrous cycle in the high-exposure females of the F_0 generation was shorter (4.2 days) and differed statistically from that of controls (5.8 days). However, the value was similar to the laboratory's historical control mean value (4.3 days) and within the historical control range (4.1-5.1 days) and not affected in subsequent generations. Hence, it is not considered to be exposure-related.

No exposure-related effects were observed on the pre-weaning body weights of the F_1 pups (PND 1-21). However, the body weights of the high-exposure F_1 pups were decreased compared to controls (by 7-7.6%, not statistically significant) during the post-weaning period (PND22-28) and the bodyweight gain in this group was statistically significantly lower than that of the controls (by 11%). A delay (approx. 2 days) in preputial separation was observed in the high-exposure F_1 males. The correlation between body weight and preputial separation in rats is clearly established; therefore, the delay in preputial separation observed in the high-exposure group of the F_1 generation is likely to be a consequence of the decrease in body weight observed in this group following direct exposure to styrene after weaning (PND22-28). The mean absolute testes weights were slightly lower in all F_1 males exposure groups by approximately 5-7%, but the observation did not follow a dose-response relationship. The differences from the controls were statistically significant for the left and right testis in the high-exposure group and for the left testis in the low-exposure group. In the absence of any associated morphological (macroscopic and microscopic) alteration and considering the lack of a dose-response relationship, the small magnitude of the decrease and the absence of such an effect in the F_0 and F_2 males, this finding is considered to be of no toxicological significance.

The general physical condition, sex ratios and survival to weaning of the F_2 generation were unaffected by maternal exposure to styrene at all levels tested. Statistically significant, exposure-related decreases in body weight of 10-13% and 7-10% were observed in the high- and mid- exposure pups of the F_2 generation respectively throughout the pre-weaning period (PND 0-21). The reductions in body weight of the F_2 pups in the high-exposure group continued throughout the post-weaning period even though exposure had stopped. No macroscopic findings attributable to exposure were evident at necropsy. Statistically significant reductions in mean absolute pituitary gland weight compared to controls were noted in the high-exposure F_2 male pups (by 34%) and in the mid- and high-exposure F_2 female pups (by 19% and 24% respectively). Also, the mean relative (to final body weight) pituitary gland weight was statistically significantly reduced in the high-exposure F_2 male pups by 22%. Other effects included statistically significant decreases in the mean absolute thymus and uterine weights by 18% of the control values in the high-exposure F_2 female pups and a statistically significant increase in the mean relative brain weight by 11% of the control value in the same group. Although the magnitude of the decreases observed in the pituitary gland weight at high- and mid-exposure levels and in the thymus and uterine weight at the high exposure is relatively large and cannot be completely accounted for by the reduced body weights observed in these pups, in the absence of information on the normal growth rate of these organs in fast-developing organisms and on its relationship to body weight development, their toxicological significance cannot be determined. However, given the lack of any associated histopathology, it is reasonable to assume that these pup organ weight reductions are unlikely to represent adverse developmental effects of styrene exposure. The attainment of the pre-weaning developmental landmarks (pinnal detachment,

surface righting response, incisor eruption and hair growth) and the acquisition of the preputial separation were also slightly delayed in the high-exposure F_2 pups. It is considered that these effects were probably due to the slight delay in growth (reduced body weights) observed in these pups.

Neurobehavioural/ neuropathological studies

Detailed functional observatory evaluations were assessed in 20/sex/group of the selected pups on PND 4, 11, 22, 45 and 60. The only finding was a statistically significant reduction in the forelimb grip strength (by 24-28% of the control values) in both sexes of the high-exposure group on PND 60. Only 6/20 male and 3/20 female individual forelimb grip strength values were outside the range of concurrent controls, and recent data obtained from the contract laboratory show that the 500 ppm group mean values for forelimb grip strength were within the range of control group means (from 8 subsequent studies) for both male and female SD rats of this age. This suggests that the observed reduction in forelimb grip strength may actually be an expression of normal variation and have no toxicological significance.

Hindlimb grip strength was also reduced by 18% of the control value in males only on PND 45. No effects were seen on PND 22, which was the earliest age of observation. Grip strength has been correlated with body weight (Maurissen et al., 2003).

Thus, the reduction in forelimb grip strength observed on PND 60 only is considered to be the consequence of the reduced body weight seen in these pups. Furthermore, as there was no similar difference in hindlimb grip strength at the same time point and no underlying histopathology, it is unlikely that the reduced forelimb grip strength represents a specific neurological effect of styrene. Chemicals that cause peripheral nerve damage typically affect both hindlimb and forelimb grip strength.

Motor activity

Motor activity in selected F_2 pups (20/sex/group) was assessed on PND 13, 17, 21 and 61 using a SDI Photobeam Activity System. Each test session lasted for 60 minutes and consisted of 12 five-minute intervals. The activity measured was the fine motor skills (i.e. grooming) and the gross motor movements. The “normal” age-related pattern of motor activity (increases between PND 13-17 and decreases between PND 17-21) appeared to be slightly shifted in the high-exposure group animals: the activities of both sexes were lower, but not statistically significantly different from those in the control group at PND 13, rose at PND 17 but did not return to expected control levels at PND 21. However, the activity in both sexes was similar to that of the control group by PND 61. Therefore, the slight shift in the age-related pattern of motor activity observed in the high-exposure group was considered to be related to the growth delay evident in this group of animals particularly in the pre-weaning stage.

Startle Response

The same animals used in the motor activity assessment (20 rats/sex/group) were used for the acoustic startle response test on PND 20 and 60 using the SR-Lab Startle Response System. The test system consisted of an isolation chamber equipped with an internal light, fan, two viewing lenses and a complete white-noise generation system. Each animal was placed in a cylindrical enclosure equipped with a motor sensor, which was then placed in the isolation cabinet. Each animal was allowed to acclimatise for 5 minutes with a background white noise. The startle stimulus for each trial was an 115dB(A) mixed frequency noise burst stimulus (20millisecs in duration) and responses were recorded during the first 100 millisecs following the onset of the stimulus. Each test session consisted of 50 trials, with an eight-

second inter-trial interval and the response data were analysed in 5 blocks of 10 trials each. The measurements obtained were: maximum response amplitude (V_{max}), average response amplitude (V_{ave}) and the time taken to obtain maximum response (T_{max}). No exposure-related trends were apparent in either sex of any of the exposed groups compared with controls.

Learning and memory

Twenty rats/sex/group were analysed for learning and memory in the Biel Maze swimming trials. Using a water-filled eight unit T-maze, animals were required to cross from one end to the other of the maze and escape by locating a platform hidden under the water surface. The time taken to swim across the maze and the number of errors for all trials were recorded. The evaluation was performed at two different ages (PND 24 and PND 62) using a different set of animals for each age and consisted of three phases conducted over seven consecutive days. The first day of testing (phase one) involved a straight channel swimming trial designed to evaluate the animals' swimming ability and motivation to escape. Each animal was placed in a straight channel opposite the escape platform and the time taken to escape recorded. Each animal was allowed four trials. Phase two (days 2-6) trials were designed to measure sequential learning (learning and short-term memory). Each animal was allowed three minutes in two trials/day for two days to solve the maze in path A and two trials/day for three consecutive days to solve the maze in the reverse path (path B). Animals failing to escape within the allotted time (3 mins) were removed and placed on the escape platform for 20 secs; then removed from the maze. The long-term memory of the animals was probed on day 7 (phase three) by challenging them to solve the maze in path A again. Biel maze data were evaluated as the mean time to escape over all trials for each of the three phases.

At PND 24, the mean time to escape in the straight channel swimming trial (day 1) was statistically significantly increased in the high-exposure male group (10.58 secs compared to 7.53 secs in controls) and a slight but not statistically significant increase was observed in the females of the same group (11.43 secs compared to 7.8 secs in control). However, no difference was seen at PND 62. For the subsequent assessments, the mean time to escape was initially relatively high and decreased throughout repeated testing in the forward path in all groups. Overall, no exposure-related differences suggestive of an impairment of learning or memory ability were observed in either sex at the two different ages tested (PND 24 and PND 62).

Swimming time in the straight channel is measured in order to evaluate basic swimming performance and exclude slower swimming times as a confounder from generalized toxicity in the interpretation of performance in the learning and memory test. Therefore, the effect on swimming ability observed at PND 24 did not represent an effect on learning and memory but it was just an indication of general malaise.

Historical control data recently obtained from the contract laboratory from a total of 20 studies in animals of the same strain and similar age show that the swimming time values in the straight channel of the 500 ppm group at PND 24 were within the historical control ranges and that the observed increase was due to an unusually low value in the concurrent controls. This suggests that this increase in swimming time may actually be an expression of normal variation and have no toxicological significance.

Overall, a temporary increase (3 sec) in the straight channel swimming time, indicative of general malaise, if any, was observed in the 500 ppm group at PND 24 but not at PND 62. This effect was consistent with the reduction in body weights, the slight delays in acquiring developmental landmarks and the shift in the normal motor activity pattern observed in this group of animals.

Neuropathology

Ten F_2 pups/sex/group were randomly selected and subject on PND 21 to brain weight measurement, morphometry and neuropathological evaluation of the brain and spinal cord. An additional 10 F_2 offspring/sex/group from the 20 used in the evaluation of motor activity were selected at random and subject on PND 72 to the same neuropathological assessments with the inclusion of the peripheral tissues. Histological and morphometric evaluations were conducted only in the control and high-exposure groups.

No notable differences were found between the exposed animals and the controls in the neuropathologic evaluations. Slight, but statistically significant decreases (by 4% of the control value) in mean brain length occurred on PND 21 in females from the mid- and high-exposure groups but the brain width and absolute brain weight were similar to control group values. Furthermore, there were no histological findings and the same finding was not reported in the male groups at either age (PND 21 or PND 72). In contrast, the mean brain lengths of females from the mid-and high-exposure groups were increased by 6.3% and 9.9% (not statistically significant) compared to controls on PND 72. Therefore, it is likely that these changes (decreases at PND 21 and increases at PND 72) in mean brain lengths of the mid- and high- exposure females are not related to parental exposure to styrene but represent an incidental finding. A slight, but statistically significant increase (by 6% of the control value) in the mean hemispheric height at Level 1 (coronal slice) was noted in the high-exposure females on PND 21. However, this finding was unaccompanied by any change in the thickness of the cortex at Level 1 or any histological change, and was not observed in the PND 72 females or in the males at either age. In view of this, the increase in mean hemispheric height at Level 1 observed in the high-exposure females is not considered to be treatment-related, but is likely to have arisen by chance.

Overall, reproductive performance and fertility were unaffected by styrene exposure in the parental generations (F_0 & F_1). Degeneration of the olfactory epithelium of the nasal cavity was observed in the high-exposure group of the F_0 and F_1 parental animals. Also, body weights were statistically significantly reduced during the pre-mating interval in the mid-exposure males of the F_1 generation (by 6-7%) and in the high-exposure males and females of both the F_0 and F_1 generations (by 6-13%). Body weights of females during gestation were statistically significantly reduced (by 6-7%) only at 500 ppm in the F_1 generation. There were no significant maternal effects on body weight at 150 ppm, although body weights of the 150 ppm F_1 females were reduced during gestation by 5-6%.

Styrene exposure caused a statistically significant decrease in body weight gain of the high-exposure F_1 pups (by 11%) and in body weight of the mid- (by 7-10%) and high- exposure F_2 pups (by 10-13%). Generally, a pattern of developmental delay was evident mainly in the F_2 high-exposure group. The delay in attaining some pre-weaning developmental landmarks (pinnal detachment, surface righting response, incisor eruption and hair growth) and in acquiring preputial separation, the decreased straight channel swimming ability, the slight shift in the normal pattern of motor activity and the reduction in forelimb grip strength observed in this study are parameters known/likely to be body weight-sensitive and hence are not considered to represent a direct and specific expression of styrene developmental neurotoxicity, but the consequence of generalised toxicity.

In conclusion, from the results of this study, 50 ppm can be identified as the NOAEC for parental toxicity based on body weight reductions at 150 and 500 ppm and on the degeneration of the nasal olfactory epithelium at 500 ppm. Based on body weight reductions and on the degeneration of the nasal olfactory epithelium at 500 ppm, the NOAEC for maternal toxicity is 150 ppm. No effects on reproductive performance and fertility were seen up to the highest tested concentration of 500 ppm. For effects on the pups, a NOAEC of 150 ppm can be established based on body weight reductions and effects on related developmental parameters at 500 ppm. Although at 150 ppm there was a decrease in pup

body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at 50 ppm. No specific developmental neurotoxicity effects were seen in the F_2 animals up to the highest tested concentration of 500 ppm.

A 3-generation study in conjunction with a 2-year continuous-exposure study has been conducted in the Sprague-Dawley derived rat (Beliles et al, 1985). Styrene was administered in drinking water at 0, 125 or 250 ppm. Consumption of styrene was estimated to be 14 (males) or 21 (females) mg/kg/day at the higher dose level. To produce F_1 pups, 10 males and 20 females were mated from each group approximately 90 days after initiation of the study. At weaning, F_1 pups were randomly selected to be mated to produce the F_2 generation and were treated as before up to about 110 days, and then mated as before to produce an F_3 generation.

Only minor general toxic effects (slight but significant reduction in bodyweight in the F_0 females after 2 years) were observed. There were no treatment-related effects on reproduction apart from a slight and not statistically significant reduction in the proportion of F_2 females producing litters at 250 ppm (75% compared with 86% in controls and 95% at 125 ppm); there was no evidence of such an effect in the F_0 and F_1 generations. Overall, this was a poorly designed study because higher dose levels could have been used. Hence, although negative results were obtained, they do not provide adequate reassurance of an absence of potential to impair fertility for styrene.

Three studies by the same authors (Srivastava et al., 1982, 1989 and 1992b) have reported testicular damage in rats at 200 and 400 mg/kg/day styrene (see section 4.1.2.6 for further details of these studies). However, a number of methodological weaknesses in the conduct of these studies put into question the reliability of these findings. It is also noted that in earlier repeated oral studies and in well conducted 2 year inhalation studies in rats at equivalent and higher doses than those used by Srivastava et al, no testicular changes or indications of any testicular effects were observed. Also, no effects on the testis and fertility parameters have been observed in a recent well-conducted OECD- and GLP-compliant rat inhalation 2-generation study with exposures up to 500 ppm (≈ 300 mg/kg/day) styrene. Therefore, despite these individual publications by the same authors reporting testicular damage, the weight of evidence indicates that styrene is not a testicular toxicant.

No evidence of any adverse effects on the female gonads has been reported in several well conducted carcinogenic and chronic toxicity studies in both rats and mice exposed via both the inhalation and oral routes to dose levels giving rise to clear evidence of toxicity and death. No signs of effects of the gonads have been reported in chronic inhalation studies in rabbits, guinea pigs or dogs at exposure concentrations giving rise to toxicity (see section 4.1.2.6).

Summary of animal studies investigating potential effects on fertility

A well-conducted two-generation inhalation study found no effects on fertility and reproductive performance in rats exposed to up to 500 ppm (2165 mg/m^3 ≈ 300 mg/kg/day) styrene, a concentration causing parental toxicity (degeneration of the olfactory epithelium and reductions in body weights).

From the other relevant studies available, there is no convincing evidence that styrene can impair reproductive performance, produce testicular toxicity, sperm abnormalities or adversely affect the reproductive organs. Thus, taken together, the data available indicate that styrene does not have the potential to impair fertility and reproductive performance in animals.

Developmental studies

Inhalation

Rats

Pregnant Sprague Dawley rats inhaled 0, 300 or 600 ppm styrene for 7 hours/day on days 6-15 of gestation (Murray et al, 1978). There were separate concurrent control groups for the two treated groups and there were 30 animals per group. Reduced maternal bodyweight gain (by 88% and 56% of the control value at 300 and 600 ppm respectively), stated to be associated with reduced food consumption (data not presented), was noted during days 6-9 of gestation. There were no significant differences in the mean number of live foetuses, resorptions per litter or mean fetal bodyweight between the groups and there was no evidence of any gross malformations. In the foetuses, there was a small (2%) statistically significant reduction in mean crown-rump fetal length at 300 ppm only. No toxicological significance is accorded to this observation since it was small and was not seen at the higher exposure level. A statistically significantly higher incidence of certain skeletal variants such as lumbar spurs and delayed ossification of sternebrae and vertebral centra, indicating delayed development, was observed at both exposure levels, but the incidences were still within the historical control ranges and hence probably of no toxicological significance. Overall, it is concluded that exposure to styrene at levels causing maternal toxicity in the rat (300 and 600 ppm) was not associated with significant effects on the foetus in this study.

In another study, 24 pregnant Wistar rats were assigned to groups and exposed to 0 (n=14), 50 (n=3) or 300 ppm (n=7) styrene vapour whole-body, for 6 hours/day on days 7-21 of gestation (Kishi *et al.*, 1992 and Kishi *et al.*, 1995). At parturition the length of gestation and number of pups per litter were recorded. Dams and pups were assessed for clinical signs of toxicity and body weight gain. On day 1 post-partum litter sizes were adjusted to 6 pups/litter. Animals were weaned at day 22 post-partum. The pups of 5 dams at 0 ppm, 2 dams at 50 ppm and 5 dams at 300 ppm were evaluated in the neurobehavioural studies over the course of the study. Pups were examined daily for development (startle reflex, eye opening, incisor eruption and vaginal patency). During the pre-weaning phase of the study (day 1 to 22) pups were also examined with respect to surface righting, pivoting locomotion, bar holding ability, negative geotaxis and cliff drop avoidance. Post-weaning (days 23 to 120) pups were examined with respect to open-field behaviour, motor-coordination, activity, operant conditioning and sensitivity to barbiturates. Dams and pups were examined histopathologically (brain, lungs, liver and kidneys): dams at day 0 (parturition) and pups on days 21 and 160 post-partum. Statistical analysis was conducted initially on a litter basis and subsequently on an individual basis.

No overt signs of maternal toxicity were observed. Maternal body weight gains, gestational lengths and the number of offspring delivered were comparable with controls. No treatment-related deaths occurred in the pups of any group. Pup body weights were statistically significantly reduced in both sexes on day 21 post-partum (by 19 and 15% of the control value at 50 and 300 ppm respectively) and in females only on day 77 post-partum (by 8 and 7% of the control value at 50 and 300 ppm respectively) but not on day 1 and 125 post-partum. Brain samples were taken from 10 pups per group on day 1 post-partum. There was no significant difference in brain weight but statistically significant decreases in certain brain neurotransmitter levels (e.g. serotonin) occurred at 300 ppm. The toxicological significance of this is unknown.

Statistically significant differences in terms of the mean litter date of eye opening, righting reflex attained, auditory startle reflex apparent and incisor eruption were reported between controls and the 300 ppm group (delays in each case were < 2 days compared to the control

values). With regard to the post-weaning neurobehavioural assessment of the pups, a statistically significantly delayed development (pivoting, bar holding, surface righting) was reported for the 300 ppm group on days 30 and 60. In some cases these differences reversed by day 120. No differences compared with controls were observed in cliff drop avoidance or negative geotaxis. Spontaneous activity was increased in pups at 300 ppm compared with controls, but no data for pups of the 50 ppm exposure group were reported. No differences in barbiturate sensitivity and no histopathological findings (in dams or pups) were observed in any test group. No historical control data were reported.

Overall, decreases in pup body weight and delays in pup development and in the acquisition of pre-weaning and post-weaning behavioural characteristics were reported in this study following prenatal exposure to 300 ppm styrene. However, it is noted that the developmental delay observed is likely to be associated with the delayed body weight development seen in these pups rather than being a direct expression of styrene neurotoxicity. It is also noted that the number of litters was too small to support any definitive conclusions and there were too few pups to assess a possible dose-response relationship. No overt maternal toxicity was reported in this study up to 300 ppm styrene.

In another study by the same workers, groups of pregnant Wistar rats (numbers not specified) were exposed whole-body to 0, 50 or 300 ppm styrene for 6 hours/day on days 6-20 of gestation (Katakura *et al*, 1999 and Katakura *et al*, 2001). Two control groups were included: a pair-fed control group (food consumption was matched with that of the 300 ppm exposed animals) and a group fed ad libitum for investigation with respect to possible nutritional effects. Implantation sites were investigated for the number of resorptions. At parturition all pups were weighed, counted and examined for external malformations. Eight pups per group (4 males and 4 females, where possible) were selected at random and were left to be reared by their natural mothers. Two pups per sex per dam were sacrificed at day 0 of parturition and the prefrontal cortex, striatum, hippocampus, hypothalamus, cerebrum, cerebellum, and midbrain were assessed with respect to levels of neurotransmitters (dopamine, 3,4-dihydroxyphenylacetic acid, 3-methoxytyramine, homovanillic acid, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and norepinephrine). At day 21 post-partum 4 pups per group (2 males and 2 females) were sacrificed and neurotransmitter levels determined. The remaining dams and pups were sacrificed at day 21 post-partum and microscopic pathology of the brain, liver, lung and kidneys conducted. Post-parturition, developmental parameters such as ear-unfolding, eye-opening, incisor eruption and righting reflex were recorded daily.

The number of litters and the number of live offspring litter were similar across all 4 groups. A statistically significant reduction (by up to 21% of the ad-lib control values) in food consumption was observed in dams exposed to 300 ppm. A slight reduction (by 8 and 4% of the ad-lib control and pair-fed control value respectively) in body weight gain was also observed in dams exposed to 300 ppm, but this did not attain statistical significance. The gestation period was increased at 300 ppm although this was only in comparison to the ad-lib fed controls; when compared to pair-fed controls there was no difference in gestation time, suggesting that the effect was related to reduced food intake.

There was a statistically significant increase in neonatal death in the 300 ppm group (7.3%) compared to both control groups (1.2% and 1.3% in ad lib and pair-fed respectively). However, when the individual litters were analysed, no significant difference was observed, indicating that the increase reported was due to a high rate of death in just one litter. Offspring bodyweight at birth was unaffected, however by day 21 post-partum a slight but statistically significant reduction in body weight was observed amongst males born to dams that had been exposed to 300 ppm styrene (by 8% of the pair-fed controls), suggesting that the effect is related to styrene exposure and not to food intake. Offspring cerebellum brain weights were similar across all groups at day 0 and day 21 post-partum.On the day (0) of parturition cerebrum weights were statistically significantly lower (by 13%) in animals of the

300 ppm group compared with ad libitum fed controls but were similar to pair-fed controls suggesting that the effect was related to reduced food intake. In the 50 ppm animals, cerebrum weights were similar to both control groups. At day 21 post-partum, cerebrum weights were reduced in the 300 ppm animals compared to the ad-lib fed controls but not to the pair-fed controls, suggesting again that the effect was related to reduced food intake.

Neurotransmitter analyses on days 0 and 21 post-partum apparently showed evidence of statistically significantly decreased levels of some neurotransmitters (homovanillic acid, 5-hydroxytrypatime and 5-hydroxyindoleacetic acid) in animals exposed at 300 ppm, compared with controls. 5-hydroxytryptamine levels were reduced in the 300 ppm animals compared to ad-lib fed controls but were similar to pair-fed controls, suggesting that the effect was related to reduced food intake. However, homovanillic acid levels were decreased in the 300 ppm animals compared to both control groups. Levels of these neuroamines in animals at 50 ppm were slightly but not statistically significantly lower compared with both control groups. Levels of other neurochemicals analysed were similar across all groups.

Delayed eye-opening, incisor eruption and air righting-reflex were all observed amongst pups at 300 ppm compared to each of the control groups. Impaired air righting-reflex was also seen at 50 ppm. However, this only attained statistical significance when compared to 'ad-lib' controls. There were no statistically significant differences between the two control groups, thus paired-feeding was concluded by the authors to have no effect on righting reflexes. Furthermore, one would expect a genuine effect on air-righting to be mirrored by a similar effect on surface-righting. This was not the case. Overall, the apparent impairment of air-righting is concluded to have arisen fortuitously and not as a result of styrene exposure. Surface righting-reflex and ear-opening were unaffected. No abnormalities were detected in the brain, lung, liver and kidney of dams or pups (at day 0 and day 21) upon microscopic examination.

Overall, slight decreases in male pup body weight on day 21 post-partum and temporal delays in the acquisition of some developmental landmarks (eye-opening and incisor eruption) were reported in this study following prenatal exposure to 300 ppm styrene. The reduced levels of a metabolite of a brain neurotransmitter were not associated with any observable histological abnormality and are considered to have arisen fortuitously or to be of no toxicological significance. No overt maternal toxicity was reported in this study up to 300 ppm styrene in comparison with the pair-fed controls.

In another study by the same workers, the possible neurobehavioural effects of styrene in rats exposed prenatally were investigated (Bingqing *et al*, 1989). Pregnant Wistar rats (number not specified) were exposed to 0, 60 or 300 ppm styrene for 6 hours/day on days 7-17 of gestation. At parturition the length of gestation and number of pups per litter were recorded. Dams and pups were assessed for clinical signs of toxicity and body weight gain. Over the duration of the study (not specified) pups (n=12 to 30) were examined with respect to physiological development (startle reaction, aerial righting reaction, timing of eye opening, incisor eruption and opening of vagina), neurological development before weaning (righting reflex, negative taxis, circumgyratory position, odour orientation, cliff avoidance and lever grasp capacity) and post-weaning behavioural tests (open-field exploration, motor coordination, activity, learning ability and continuous reinforced learning). No experimental details of the studies conducted were reported.

No overt signs of maternal toxicity were observed. Maternal body weight gains, gestational lengths and the number of offspring delivered were similar to controls. No treatment-related deaths in the pups of any group were reported. Pup body weights in the high exposure group (300 ppm) were reported to be statistically significantly decreased on day 1-7 post partum compared to controls, and were significantly decreased in animals of both exposure groups at days 21, 77 and 125. The magnitude of the body weight changes was not reported.

With respect to physiological and neurological development no differences were observed in the low exposure group animals (60 ppm) compared to controls. In the high exposure group animals (300 ppm) it is reported that eye opening, incisor eruption and aerial righting response all occurred later than in controls (no data presented), and that negative taxis and circumgyration test results showed a delay of 8-12 days, and the lever grasp test a delay of 4-12 days. No significant difference was evident in the righting test or the cliff avoidance.

With regard to the post-weaning assessments, in the open-field test the activities of the pups of the low exposure group (60 ppm) were similar to controls. Pups of the high exposure group (300 ppm) at age 30-31 days and 60-61 days were statistically significantly more active compared with controls. However, activities were similar by days 127-128. It was reported that at days 30 and 60 the results of the on-rod tests showed an effect of maternal exposure to 300 ppm styrene (no data were presented). Spontaneous activity (exploration and total activity) was increased in pups of the high exposure group compared with controls. No differences in manipulative behaviour were observed between exposed and control animals. No histopathological investigations were reported.

Overall, the study appears to suggest that pups exposed prenatally to 300 ppm styrene showed temporary and reversible differences in developmental responses; no such effects were seen in pups exposed to a lower exposure concentration of 60 ppm. However, given the lack of experimental and statistical detail and the absence of data in the report, no firm conclusions can be drawn from it.

In a poorly reported study, rats were exposed for 4 hours/day, 5 days/week to 167 ppm styrene on gestation days 2 to 16 or to 47 ppm of styrene on days 2 to 21 (Vergieva et al, 1979). Dams were allowed to litter, with the exception of some animals exposed to 47 ppm, which were sacrificed on day 21. The number of animals per group was small (7-12). Maternal effects were not reported for those sacrificed on day 21; in these animals there were no effects on preimplantation or postimplantation death or on fetal malformations. In dams allowed to litter, there were no treatment-related effects on pup weight. At 30 days after the end of exposure, the number of erythrocytes in dams and offspring was higher in the 47 ppm group than in controls, but this parameter was not measured in the higher dose group. At 167 ppm, but not at 47 ppm, haemoglobin concentration was significantly higher than in controls. There is no clear biological significance to these findings, particularly since such effects have not been observed in other studies. The value of this study was also limited by the small numbers in the groups and by the confused and poor reporting. No significance can be accorded to the findings.

In a limited post-natal study, THA strain rat dams and pups were exposed whole-body to 0, 25 or 50 ppm styrene for 7 hours/day from day 1 after birth throughout lactation to weaning and then to day 48 of age (Shigeta et al, 1989). Eight offspring were assigned per dam after birth (3-5/sex). In total 25 male pups and 17 female pups were exposed to 25 ppm and 14 males and 17 females were exposed to 50 ppm. A control group comprised 12 animals, males and females. Bodyweight of offspring was recorded weekly. The times of initiation of pinna detachment, incisor eruption and eye opening were also recorded. Behavioural tests (open-field activity at 9 weeks of age and Sidman avoidance test at 7 weeks of age) were evaluated in the pups. No treatment-related effects in the dams were observed. Body weights of pups in the control and exposure groups were not equivalent at the start of the study, with body weights of animals in the exposure groups being statistically significantly less than controls at day 1 (by 12%). This difference in body weights persisted throughout the study and indicated that the control group may have already been further advanced in development at the start of the study. Delays in pinna detachment and incisor eruption were observed in both treated groups but there was no dose relationship. There was no effect on eye opening. A statistically significant difference from control values was also noted in the 50 ppm group in one of the behavioural parameters measured using the open field test. Overall, although minor differences from control values were observed in styrene-exposed

animals, there was no apparent dose-response relationship. Given the differences between the control and test animals at the start of the study (the controls were more advanced) and that this difference was maintained throughout the study, it is possible that this is the explanation for the differences observed.

A poorly reported two-part study investigating the developmental toxicity of styrene is available which reports an increased incidence of embryonic, fetal and neonatal deaths in rats following inhalation exposures of between 0.4 and 12 ppm styrene for 4 hours per day throughout pregnancy or for the first trimester only (exact duration of exposure not reported) (Ragul'ye, 1974). However, the reporting of the study is limited in terms of the quantitative information presented and the statistical significance of the findings. In most instances it is unclear which test group (1 or 2) was exposed to which concentration of styrene and presented which effects; therefore clear interpretation of these results is impossible. Also, no details of maternal health were reported for any animals in the study, although limited investigations were apparently conducted. No adverse gross or microscopic findings were observed in animals from either exposure group. Overall given the poor reporting of this study and the absence of information pertaining to maternal health, no conclusions regarding the developmental toxicity of styrene can be drawn from it. Furthermore, given the low exposure concentrations apparently used, it is difficult to reconcile these findings with those from other better-reported studies, where no such mortality effects were observed at appreciably higher exposure levels.

Other species

Groups of mice were exposed to 250 ppm styrene for 6 hours/day on days 6-16 of gestation (Kankaanpaa *et al*, 1980). The study report was only brief. Control mice were sham-exposed and produced 15 litters. In the treated group there were 13 litters. The pregnant females were sacrificed on day 16. No information was given as to whether any signs of toxicity were observed in the maternal animals but some toxicity was likely as preliminary studies had shown 500 ppm to be lethal to 2 out of 6 pregnant mice, using a similar exposure regime. The mean number of live fetuses per litter was similar in styrene-exposed (5.8) and control (6.3) groups. A small difference in the number of dead or resorbed foetuses per number of live foetuses was observed; the results were 21/115 (18%) for controls and 28/104 (27%) for exposed animals. The data presented for this effect are unconvincing; data on dead or resorbed implants per litter should have been presented. There was no evidence of any gross malformation, but there was an increased incidence of minor skeletal variations, rib fusion and extra ribs. Only the total "abnormality" figures were given (2.9% in treated, 0.9% in controls) and it is not possible to interpret such aggregated and poorly described findings. Overall, because of the limitations in the analysis and reporting, no useful conclusions can be drawn from this study.

Groups of 18-19 mated ICR female mice were exposed, whole body, to 0, 2, 20, and 100 ppm (0, 8.7, 87, 433 mg/m^3) styrene vapour continuously between days 0 and 15 of gestation (Ninomiya *et al*, 2000). The number of implantations, resorptions, number of foetuses and number of dead or deformed foetuses were recorded. In addition, histological examination of the liver, kidney, spleen, lungs, ovaries, and placenta was conducted in the dams. Live foetuses were removed, examined visually and weighed.

No adverse effects were seen in the non-pregnant females exposed to styrene under the same conditions. At the highest exposure level the dams showed signs of hyper-activity and reduced bodyweight gain (45% lower than controls). There were no mortalities. Dams exposed to 100 ppm (433 mg/m^3) also showed reduced amounts of adipose tissue, and lower liver, kidney and spleen weights (26%, 12% and 41% respectively) when compared to controls. The fertility index (percentage of mated animals that became pregnant) was not statistically-significantly affected (fertility index 18/19, 16/18, 15/18, 14/18 amongst mice at 0,

2, 20, 100 ppm respectively). There were no substance-related effects on the number of implantations, resorptions, or live foetuses. Reduced placental weight was noted amongst animals exposed to 100 ppm (mean 0.09g compared to 0.11g in controls). Reduced foetal weight was also noted at 100 ppm (mean 0.36g compared to 0.48g in controls). Overall, the only signs of reproductive toxicity were evidence of reduced placental and reduced foetal weight at levels of exposure (100 ppm for 24h/day) associated with a marked impairment of maternal growth.

A developmental toxicity study has also been carried out in Chinese hamsters, dose levels of 300-1000 ppm being used (Kankaanpaa et al, 1980). Again, few details were given in the report. Exposures were for 6 hours/day on days 6-18 of gestation and animals were killed on day 18. There were 15 litters in the control group but only up to 7 litters per exposed group. A marked reduction in litter size was noted in the group of 7 litters at 1000 ppm; the number of dead or resorbed implants was significantly higher (33/50, $p<0.001$) at this concentration. However, no signs of any toxic effects on the developing fetus were noted at concentrations up to and including 600 ppm. Even though 1000 ppm is a relatively high concentration, no details of maternal toxicity were presented. The number of litters was very small (2-5) in the other exposed groups and therefore the study cannot be regarded as adequate. Overall, it is difficult to draw firm conclusions as to the interpretation of either the positive and negative findings seen in this study at the various exposure levels used.

New Zealand rabbits (20 per group) were exposed via inhalation on days 6-18 of gestation to 0, 300 or 600 ppm styrene for 7 hours/day (Murray *et al*, 1978). No evidence of maternal toxicity was found. The were no significant differences in the mean number of live fetuses, resorptions per litter or mean fetal bodyweight between the groups and there was no evidence of any gross malformations. Skeletal variants (unossified sternebrae) were statistically significantly increased at 600 ppm only; data were not reported in detail but the authors stated that the results were within the historical control range. Overall in the rabbit no evidence of maternal toxicity was observed at exposures of up to 600 ppm and there were no exposure-associated effects on the foetus.

Oral

Rats

In the oral-dosing part of the Sprague Dawley rat study by Murray *et al* (1978) described above, no adverse effects were noted on the developing fetus when pregnant rats were given doses of 0, 90 or 150 mg/kg, twice daily (i.e. 180 or 300 mg/kg/day), on days 6-15 of gestation. There was one negative control group (39 animals); treated groups comprised 29 or 30 dams. Reduced maternal bodyweight (by 33% and 39% at 180 and 300 mg/kg respectively) was again associated with decreased food intake on days 6-9 in treated groups.

Groups of 13 treated and 30 control Sprague-Dawley rats were administered 1147 mg/kg/day styrene on days 6-15 of gestation (Chernoff *et al*, 1990). Maternal toxicity was observed as a statistically significantly reduction in bodyweight gain (by 26%) compared with controls. There was no effect on fetal weight, death, skeletal or brain morphology or other malformations but an increase in the fetal anomaly, dilated renal pelvis, was reported. Full details were not made available in the report but it can be concluded that the only effect on the foetus at a maternally toxic dose (1147 mg/kg/day) was an increased incidence of a foetal anomaly; there were no abnormalities.

Styrene has been used as a negative control in a developmental toxicity study in the Sprague Dawley rat (Daston *et al*, 1991). Groups of animals were administered 0 or 300 mg/kg styrene in corn oil by gavage on day 11 only. Animals were sacrificed on day 20. Maternal toxicity consisted of significant reductions in bodyweight and food consumption on the day after dosing. No developmental toxicity was observed in the 8 litters from styrene-treated dams. There were 7 vehicle control litters. Although the protocol for this study is not rigorous, it is nevertheless a useful addition to the database.

A study involving oral administration of styrene at 0, 250 or 400 mg/kg/day on days 6-15 of gestation in albino rats was briefly reported (Srivastava *et al*, 1990). The number of litters was 14 in controls, 10 at 250 mg/kg and 12 at 400 mg/kg. Maternal body weight gain was statistically significantly reduced (by 16% at day 20) in the 400 mg/kg/day group only. The only effect reported to be statistically significant was reduced fetal weight (22% decrease) at 400 mg/kg/day. Non-statistically significant differences observed between the 400 mg/kg/day group and the controls were increased resorptions (12% vs 1%), decreased implantations (70 vs 132) and reduced live fetuses per litter (4.8 vs to 9.4). No fetal malformations were observed. Overall, in this study, reduced foetal weight and increased embryonic/foetal death occurred at a dose level (400 mg/kg/day) associated with maternal toxicity.

In a very briefly reported study, groups of 4 female albino rats were treated orally with 0, 200 or 400 mg/kg/day styrene in groundnut oil each day during lactation (Srivastava *et al*, 1992a). Each dam was randomly assigned 8 male pups at day 0 (parturition). At 21 days, 6 pups per group were randomly chosen from each dose group and sacrificed at 31, 61 and 91 days respectively, and the testes and epididymides removed and weighed. A portion of one testes was homogenised for analysis of enzymic activities (sorbitol dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, acid phosphatase, γ-glutamyl transpeptidase and β-glucuronidase); the remaining testis was examined histopathologically. The epididymal spermatozoa were counted.

During the study, no signs of toxicity or deaths were observed in either the dams or pups of any dose group. Pup body and testes weights from both dose groups were comparable with controls throughout the study. In pups of dams receiving 200 mg/kg/day, throughout the study, there were no adverse histopathological findings in the testes, and spermatozoa counts and enzymatic activities were similar to controls. At 400 mg/kg only, mean sperm counts were reduced by approximately 20% (data were presented graphically only) at both days 61 and 91 (data for day 31 were not reported). There were also some apparent statistically significant alterations in some testicular enzyme levels; sorbitol dehydrogenase levels were decreased at day 61 by 11% compared with controls, but at days 31 or 91 levels were similar to controls. Lactate dehydrogenase levels were increased by 20 and 15% at days 31 and 61 respectively compared with control levels, but were similar to controls at day 91. Acid phosphatase levels were increased by 19 and 14% at days 31 and 61 respectively compared with control levels, but were comparable with controls at day 91.

The reporting of this study is limited and, in the absence of information on the normal ranges in such young rats of the testicular enzymes examined, the toxicological significance of the differences (in each dissection) observed between test and control groups is difficult to assess. It is noted that, in contrast to the previous investigation (by the same workers) in which maternal toxicity was observed at 400 mg/kg/day, no overt signs of maternal toxicity were reported at the same dose level in this study. The sperm count data are inadequately presented and unconvincing. Furthermore, when these results are compared with repeated oral studies and also with those of well conducted 2 year inhalation studies in rats at equivalent and higher doses, in which no testicular changes or indications of any testicular effects were noted, these reported findings appear to be unreliable. Overall, no reliable conclusions can be drawn from this study.

Khanna et al (1991) have reported that eye-opening and fur growth were delayed in pups (n=15) born to female Wistar rats receiving a “low” protein diet containing either 0 or 100 mg/kg/day styrene on days 6-15 of gestation, but not in pups of dams fed normal diet containing the same does level of styrene. These effects were reported as being ‘marginal’ in the pups of dams fed with “low protein” without styrene but more apparent in those receiving styrene. The body weight gain of pups born to styrene-exposed dams fed a “low” protein diet was decreased by 27% compared with non-exposed/normal protein-fed animals. The pups of “low” protein fed dams (styrene exposed and unexposed) also showed a delayed development of the surface and air righting reflex, of the cliff avoidance response and a marginal increase in the activity of monoamine-oxidase, succinic dehydrogenase and Na^+/K^+ ATPase and in the levels of dopamine and serotonin receptors in the brain, compared with pups of dams fed normal diet. Overall, this study adds little to the hazard assessment of styrene; many of the effects seem to be related more to the unusual diet received by the animals.

A study is available which was designed principally to investigate the effect of styrene on the dopamine receptors in pre and postnatally exposed rats (Zaidi *et al*, 1985). Pregnant dams (numbers not specified) were administered 0 or 200 mg/kg styrene in groundnut oil by oral gavage from day one of gestation to parturition. At parturition pups were randomised and litter sizes adjusted to 8 pups per dam. Animals were then divided into 4 exposure groups each containing 3 litters: group A comprised control dams and their natural pups (controls); group B comprised styrene-exposed dams and their natural pups (gestation and lactation exposure); group C comprised control dams and fostered in utero styrene-exposed pups (gestational exposure only); and group D comprised styrene-exposed dams and fostered unexposed pups (lactational exposure only). Treatment of the pups and dams was then continued accordingly until week 3.

Average litter weights and number of pups were recorded both at parturition and at week 3 post-parturition. Behavioural studies (not specified) were carried out on 8 pups/group at week 3. Measurements of amphetamine-induced locomotor activity and apomorphine-induced stereotypy were also taken as parameters of dopamine receptor sensitivity. Six pups/sex were then sacrificed and the brains removed and the corpus striata dissected for assessment of dopamine receptor binding by measuring the binding of ^{3}H-spiroperidol, as a specific ligand.

No significant difference in the number of pups per litter or in average body weights between control and exposed pups were observed at either time point. No overt signs of maternal toxicity were observed during the study. No evidence of any significant treatment-related effect on the protein content of the striatal region of the brain was observed at dissection. In pups exposed during gestation only there was no effect on ^{3}H-spiroperidol binding compared with controls. However, in pups exposed during gestation and lactation or during lactation only the binding of ^{3}H-spiroperidol was statistically significantly increased compared with controls (both by 20-26%). Further analysis revealed the increased ^{3}H-spiroperidol binding was due to an increased number of dopamine receptors; there was no change in binding affinity. Pups exposed during gestation and lactation, or during lactation only, also showed a significant increase in amphetamine-induced locomotor activity and apomorphine-induced stereotype. These data suggest that exposure to styrene may alter brain dopamine receptor levels during maturation (days 7 to 28 post partum) in the rat. The toxicological significance of this finding in relation to human health is unknown.

In vitro

In a briefly reported study, on day 12 of gestation pregnant Sprague Dawley rats (number not specified) were sacrificed, the gravidi uteri removed and cell cultures of embryonic mid brains (CNS) and forelimb buds (LB) prepared (Gregotti *et al*, 1994). Cultures were then

treated with styrene at concentrations of 100-300 µg/ml, both in the presence and absence of exogenous metabolic activation and incubated at 37°C for 5 days prior to staining. No adverse effects on viability of differentiation of LB cultures at any styrene concentration were observed. No concentration-related changes in styrene-treated CNS cultures were observed in either the presence or absence of metabolic activation, compared with untreated controls. No evidence of cytotoxicity was observed. However, since this is a non-validated test system, no conclusions can be drawn from these results with respect to any potential developmental toxicity that styrene might possess.

Summary of developmental toxicity studies in animals

Data from inhalation and oral developmental toxicity studies in a number of species are available, but most are either poorly designed or reported. There are no studies using the dermal route of exposure.

In the rat, inhalation exposure produced no evidence of significant effects on conventional parameters assessed in the foetus at non-maternally toxic exposure concentrations of up to 600 ppm styrene. However, developmental delays have been reported postnatally in a number of non-GLP, non-OECD studies at 300 ppm styrene in the absence of overt maternal toxicity. This has been confirmed by a recent, well conducted two-generation study in which, a pattern of developmental delay (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength), including decreased body weights, was evident mainly in the F_2 pups of the high exposure group (500 ppm). It is noted that, in contrast to previous investigations, in this OECD- and GLP-compliant study the delay in pup development was seen at an exposure level causing some maternal toxicity (reductions in body weights of 6-7% and degeneration of the nasal olfactory epithelium). No specific developmental neurotoxicity was seen in this study up to the highest tested concentration of 500 ppm.

Other species investigated using the inhalation route of exposure included the mouse, rabbit and Chinese hamster. No styrene exposure-associated effects were observed in the rabbit study. The majority of the studies conducted in mice suffer from many limitations and so, no conclusions can be drawn from them. In a recent study, reduced placental and foetal weights were observed at an exposure level (100 ppm) associated with impairment of maternal growth. Significantly higher numbers of dead or resorbed implants were observed at 1000 ppm in the Chinese hamster study. However, in the absence of information on maternal toxicity, which is most likely to have occurred at such a high exposure concentration, no firm conclusions can be drawn from the results of this study.

Only studies in the rat are available using the oral route of exposure; generally no significant effects on any of the conventional parameters assessed in the foetus were seen at dose levels up to 250 mg/kg/day, at which maternal toxicity was observed. In one study, an increased incidence of a fetal anomaly (dilated renal pelvis) associated with a significant reduction in maternal body weight was observed following the administration of 1147 mg/kg/day styrene. Fetotoxicity (reduced foetal weight and increased embryonic/foetal death) was also reported at 400 mg/kg/day in another study, but this again was in the presence of maternal toxicity. An increase in the number of brain dopamine receptors in rat pups exposed to 200 mg/kg/day styrene during gestation and lactation or during lactation only was reported in one study, however, the toxicological significance, if any, of this finding is unknown.

Overall, it can be concluded that styrene does not cause developmental toxicity in animals as evaluated by structural endpoints at inhalation exposures of up to 600 ppm and oral exposures of up to 250 mg/kg/day and by neurological endpoints at inhalation exposures of up to 500 ppm. However, reduced pup growth and pup developmental delays (delays in

attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength) were seen postnatally in rats at exposure levels (300-500 ppm) causing, in some cases, maternal toxicity. With the exception of a small reduction (up to 10%) in pup body weight, no developmental effects were observed at 150 ppm (≈120 mg/kg/day) in a well-conducted 2-gen study.

Taking into account all of the available information, it is suggested that 150 ppm is taken forward to the risk characterisation as the NOAEC for potential effects of styrene on development. Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. This information will be taken into account when judging the adequacy of the Margins of Safety in the risk characterisation.

Summary of reproductive toxicity studies in animals

A well-conducted two-generation inhalation study found no effects on fertility and reproductive performance in rats exposed to up to 500 ppm (2165 mg/m^3 ≈ 300 mg/kg/day) styrene, a concentration causing parental toxicity (degeneration of the olfactory epithelium and reductions in body weights).

From the other relevant studies available, there is no convincing evidence that styrene can impair reproductive performance, produce testicular toxicity, sperm abnormalities or adversely affect the reproductive organs. Thus, taken together, the data available indicate that styrene does not have the potential to impair fertility and reproductive performance in animals.

Data from inhalation and oral developmental toxicity studies in a number of species are available, but most are either poorly designed or reported. There are no studies using the dermal route of exposure.

In the rat, inhalation exposure produced no evidence of significant effects on conventional parameters assessed in the foetus at non-maternally toxic exposure concentrations of up to 600 ppm styrene. Pup developmental delays have been reported in a number of non-GLP and non-OECD studies at 300 ppm styrene in the absence of overt maternal toxicity. This has been confirmed by a recent, well conducted two-generation study in which, a pattern of developmental delay (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength), including decreased body weights, was evident mainly in the F_2 pups of the high exposure group (500 ppm). It is noted that, in contrast to previous investigations, in this OECD- and GLP-compliant study the delay in pup development was seen at an exposure level causing some maternal toxicity (reductions in body weights of 6-7% and degeneration of the nasal olfactory epithelium). No specific developmental neurotoxicity was seen in this study up to the highest tested concentration of 500 ppm.

No convincing evidence of specific developmental effects was seen in other species (mice, rabbits and hamsters) exposed to styrene via the inhalation route of exposure.

Only studies in the rat are available using the oral route of exposure; generally, no significant effects on any of the conventional parameters assessed in the foetus were seen at dose levels up to 250 mg/kg/day, at which maternal toxicity was observed.

Overall, it can be concluded that styrene does not cause developmental toxicity in animals as evaluated by structural endpoints at inhalation exposures of up to 600 ppm and oral exposures of up to 250 mg/kg/day and by neurological endpoints at inhalation exposures of up to 500 ppm. However, reduced growth and developmental delays (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength) were seen postnatally in rats at exposure levels (300-500 ppm) causing, in some cases, maternal toxicity. With the exception of a small reduction (up to 10%) in pup body weight, no developmental effects were observed at 150 ppm (≈120 mg/kg/day) in a well-conducted 2-gen study.

Taking into account all of the available information, it is suggested that 150 ppm is taken forward to the risk characterisation as the NOAEC for potential effects of styrene on development. Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. This information will be taken into account when judging the adequacy of the Margins of Safety in the risk characterisation.

4.1.2.9.3 Studies in humans

Fertility

Menstrual disorders were investigated in women working in the reinforced plastic industry in the USA (Lemasters *et al,* 1985). There were 174 exposed and 449 unexposed workers in the analysis. Exposed subjects were divided into two categories: higher exposures were estimated to be “mean 52 ppm” and others “mean 13 ppm” (further details not given). Exposed and unexposed groups were not significantly different when incidences of abnormalities including severe dysmenorrhea and intermenstrual bleeding were compared. Many factors known to influence menstruation were included in the multiple regression analysis. Exposure to styrene was not associated with disorders.

The control incidences of menstrual abnormalities reported by Lemasters *et al* (1985) were similar to or higher than incidences claimed for styrene-exposed workers in Russian studies. The latter are of poor quality and therefore not discussed further here (Bondarevskaya, 1957; Bondarevskaya, 1961; Zlobina *et al*, 1974; Zlobina *et al*, 1975). It is considered that these studies are not useful in contributing to the toxicological picture for styrene.

In a briefly reported study, no significant difference in menstrual cycle characteristics (duration of cycle, days and quantity of bleeding) were apparently found in a cohort of 110 women occupationally exposed to benzene, toluene, xylene and styrene, compared with 45 unexposed controls from the same plant (Georgieva *et al*, 1998). However, given the limited reporting, no reliable conclusions can be drawn from this report.

The time to pregnancy for the partners of men occupationally exposed to styrene has been investigated in a European multicentre study for which preliminary results are available (Kolstad *et al*, 1999a). The study population was drawn from three Danish, four Italian and 14 Dutch companies producing a range of products. A total of 1479 men were identified as employed during the period 1995-1997 and of these 1009 (68%) underwent interview or answered a postal questionnaire. From these 643 were included in the study having been identified as married and having fathered a child. Men who reported a contraceptive failure

(42) or did not recall the time to pregnancy (35) were excluded. A further 25 were also excluded as they had fathered a pregnancy less than 13 months prior to the date of data collection. This was in order to ensure a similar observation period for all study subjects because the authors censured removed from study all participants after 13 months of waiting time to pregnancy, to exclude the possible effects of medical intervention for infertility. Thus overall 541 men (of whom 188 were exposed to styrene) were included and the focus was on the time to pregnancy for the youngest child. Measurements (720 in total) of styrene in workplace air were also available for three Danish and five Dutch companies collected between 1960 and 1995, largely by charcoal absorption tubes. Assessment of urinary mandelic acid levels was also made for a subgroup of 28 workers, which correlated with frequency of laminating or moulding reinforced plastics goods and the use of protective equipment. Using the information available on exposure potential (airborne measurements, mandelic acid levels, work pattern and use of protective equipment) the styrene-exposed workers were separated into unexposed, low, medium and high exposure categories. All pregnancies occurring before employment in a reinforced plastics industry were classified as unexposed. Fecundability ratios were calculated as unadjusted or adjusted using regression modelling to take into account exposure variables, time-to-pregnancy starting date, study centre, maternal age, parental smoking habits, oral contraceptive use and parity.

Of the 541 men studies, 478 (87%) fathered pregnancies with 13 months, 87% (308/353) in the unexposed and 88% (166/188) in the styrene-exposed workers. Unadjusted fecundity ratios showed a slight borderline statistically significant trend with exposure. Adjustment for all factors except study centre did not alter this trend but inclusion of study centre indicated no association between styrene exposure and fecundability. Moreover, the Italian workers, who constituted a large proportion of the high exposure group, reported an overall fecundability ratio which was 50% higher than that of the Danish and Dutch workers and no exposure response relationships were seen within each participating centre. Overall, these preliminary results from a European multicentre study showed no effects of styrene exposure on male fecundity as assessed by time to pregnancy of the partners.

The preliminary results from a study of semen quality and sperm chromatin structure in Danish styrene-exposed workers are available (Kolstad *et al*, 1999b,c). The workers were drawn from the same Danish factories used in the study on fecundity described above. Of 131 workers employed between October 1994 and February 1995, 37 (30%) agreed to participate on starting employment, with 3 then being excluded due to previous styrene exposure, vasectomy or previous welding exposure, which the authors considered to be a potential contributor to reduced semen quality. During the following 6 months a further 11 of the workers were lost to follow-up because of leaving the companies or for other unspecified reasons. The remaining 23 workers provided semen samples during their first work week and then after 6 months at work, apart from two who provided second samples at 2 or 3 months respectively. Measurements made of semen quality were: semen volume, sperm density, total sperm count (product of sperm density and semen volume), sperm motility and sperm morphology including staining for vital and nonvital sperm. Flow cytometry of sperm was also performed in order to quantify fluorometrically the degree of acid-induced denaturation of DNA to assess chromatin vulnerability. Information on a number of days of sexual abstinence prior to semen sampling, a period of fever 3 months prior to sampling, the occurrence of spillage during sampling and time between sampling and measurement was recorded. For an external comparator, 21 farmers who had been examined using an identical study protocol in another study were included. For each plastics worker on farmer was selected at random among 248 eligible farmers after matching for season to take account of regional changes in semen quality. For the two workers who provided the second sample within the same season (2 or 3 months after the first) no external referents were used. The farmers were recruited from the same rural area as the plastics workers but were on average older (39 as opposed to 27.5 years) and 5 were smokers compared to 14 smokers in the plastic workers group; average alcohol consumption was similar in both

groups. Post-shift urinary mandelic acid was assessed in the plastics workers, as a measure of styrene intake, for five successive days just after hiring and again during 5 additional days after six months at work.

A subgroup of workers exposed to styrene taken from those monitored for organic solvent exposure by the Finnish Institute of Occupational Health was studied (Taskinen *et al,* 1989). Pregnancies of wives of male workers were traced from national registers. Questionnaires were used to identify paternal (preceding pregnancy) and maternal exposure (first part of pregnancy). Cases of spontaneous abortion were matched with three referents. The odds ratio for paternal exposure to styrene was not significantly increased. Maternal exposure to individual solvents was not assessed; the odds ratio for exposure to organic solvents was not increased.

Sperm density and total sperm count were found to be lower (median levels by about 30% for sperm density and 50% for total count) six months after the start of factory work compared to at the time of starting work. A slight reduction in the proportion of normal sperm (44% compared to 38%) was also seen. In contrast, the proportion of nonvital sperm was lower at six months compared to the start of exposure and the sperm velocity was increased. Allowing for change in abstinence time, fever, spillage and latency to examination in a multiple regression model did not affect these findings. Only small changes in these parameters were seen for farmers. However, when plastics workers were compared to the farmers with respect to individual semen parameters, a statistically significant lower value in styrene-exposed workers was seen for sperm density only, and not for any of the other parameters. The differences in individual semen parameters were also compared to the post-shift urinary mandelic acid measurements. No indication of any exposure response relationship was seen between the mandelic acid level and any of the semen parameters, apart from the proportion of nonvital sperm, which increased with increasing mandelic acid concentration with a borderline statistical significance when tested for trend. However the trend was due entirely to a decreasing proportion of nonvital sperm in the nonexposed and low-exposure workers, whereas essentially no increase was seen in the higher exposure categories. No change was seen in acid-induced denaturation of chromatin in styrene-exposed workers over the time of the study, although an indication of a weak increase was seen in association with mandelic acid levels. These preliminary results have made some associations between work involving exposure to styrene and changes in some semen parameters. However, no association was found between a measure of styrene intake (mandelic acid) to styrene and changes in these parameters. Overall, therefore, no firm conclusions can be drawn from this study with respect to whether or not styrene affected semen parameters in these exposed workers.

Another study on semen quality in GRP manufacturing in Denmark, is available (Jelnes, 1988). Breathing zone median styrene levels were given as 70, 86, and 131 ppm (reference period not given), representing samples taken at 3 different times, with peak levels reportedly two-fold higher. Semen and blood samples were collected from 25 men. An age-matched control group was drawn from men attending an infertility clinic for the first time. It is therefore possible that semen parameters in controls would be poor compared with those in the general population. The controls were not matched for other possible confounding factors. There were no differences between the groups when serum luteinising or follicle stimulating hormones, semen volume or sperm count were measured. In the styrene-exposed group, the proportions of live sperm and motile sperm were higher but percentage of abnormalities in sperm heads was also statistically significantly increased. The study is of a poor design because of the choice of reference group, the use of only single sampling and the relatively small group size. No firm conclusions can be drawn.

One report from USSR concerning sexual problems in male GRP workers is not useful with respect to styrene exposure (Neshkov and Nosko, 1976).

Developmental toxicity

Spontaneous abortions

A few studies have been carried out in Finland to determine any alerts for spontaneous abortion in female chemical workers (Hemminki *et al*, 1980 and Hemminki *et al*, 1984). The studies were based on abortion data collected in a unique extensive hospital register in Finland. Potential exposure to chemicals was only indicated tentatively by using union membership as a surrogate. There was a higher rate of spontaneous abortion in the chemical workers than was found in the general Finnish population. On subdividing chemical workers by industry, this finding remained true for several subgroups including workers in styrene production and use. However, on extending the follow-up period of the original study, an increase in spontaneous abortion rates in styrene workers was not confirmed. Comparing spontaneous abortion rates during periods of union membership with periods of non-union membership (using the women as their own controls), the spontaneous abortion rates were higher during non-membership. The nature of this study is such that it is not possible to draw conclusions with any confidence, because of lack of information on exposure, possibility of confounding factors not being account for and the ambiguous nature of the results.

In a poorly reported and poorly analysed study comparing 67 female lamination workers with a reference group of food and textile workers, no difference in spontaneous abortion rate was observed between the exposed subjects and the referents (Harkonen and Holmberg, 1982). The exposed subjects had a higher rate of spontaneous abortion while exposed to styrene than the same group, used as internal controls, before they commenced work with styrene. However, the relevance of this finding is very doubtful since a similar effect was seen in the reference population on comparing the same time periods. The study was of low power and overall, the findings are inconclusive.

There was a small case-control study carried out in Finland (Lindbohm *et al*, 1985 and (Lindbohm *et al*, 1990). Potential exposure to styrene was assessed by membership of the chemical workers union. In polystyrene plastics workers, odds ratios for spontaneous abortion were low (<1). However, the authors acknowledge that the study had little power for detecting such effects.

Data from a large survey in Montreal were used to study spontaneous abortions in pregnancies in which women had worked in plastics factories (McDonald *et al*, 1988). The survey was conducted from 1982 to 1984 by interviewing 56,012 women patients in hospital after delivery or spontaneous abortion. Information on past pregnancies was included and employment coded against a standard Canadian classification. Some early abortions not treated in hospital may not have been included. The total number of pregnancies covered was 104,620. Supplementary questions on occupational exposure and ergonomics were asked. Occupational hygienists followed up selected cases and controls. Initially 222 pregnancies were identified where the women was apparently employed for at least 30 hours/week in plastics plant from the time of conception. The workplace was then traced where possible and the manufacturing process determined. There were 26 pregnancies and 5 spontaneous abortions designated “polystyrene work alone” and 50 pregnancies and 13 spontaneous abortions involving “mixed including polystyrene” exposure. All the processes involved polystyrene or expanded polystyrene; none were reinforced plastics. A group of 39 women worked in administrative roles at the same plants. Two spontaneous abortions occurred in this control group. Logistics regression for 7 potentially confounding factors (age, gravidity, history of abortion, ethnic group, educational level, smoking habit, alcohol consumption) in all working women (47,316 pregnancies) was used to calculate the expected numbers of spontaneous abortions. The ratio of observed to expected

spontaneous abortions was 1.58 (90% confidence intervals 1.02-2.35) for the 76 pregnancies (18 observed) in the styrene-exposed group. This means that after confounding factors were corrected for, the ratio for spontaneous abortion just reached statistical significance at the 10% but not the 5% level. From these data alone, it is not possible to deduce whether or not this indicates a true effect. In summary, it is generally recognised that studying the endpoint of spontaneous abortion in humans is difficult. The studies available, looked at isolation, provide no convincing evidence of whether or not styrene, is able to produce this effect in humans.

Congenital abnormalities

In Finland, a series of studies looked at cases of congenital malformation against occupational exposure.

Two cases have been reported of CNS defects in infants whose mothers had been exposed during pregnancy to chemicals in the reinforced plastics industry in Finland (Holmberg, 1977 and 1978). On the basis of a gross estimate of the number of pregnancies expected in Finnish women workers in that industry, over the time period of the two cases, it was estimated that the two cases represented a significant excess over that expected. However, in reality, it is not possible to draw any conclusions from the two isolated cases reported. In further studies over a number of years, the apparent initial excess in CNS defects was not confirmed and other types of malformation were not increased in cases where there was maternal exposure to styrene (Holmberg, 1979, Holmberg 1982, Kurppa *et al*, 1983 and Holmberg *et al*, 1986). However, the population exposed to styrene in these studies was too small for this to represent an adequate investigation of this endpoint.

Another Finnish study looked at malformation rates in children born to male and female workers exposed to styrene in the GRP industry (Harkonen *et al*, 1984). Data came from national registers. There were 1,698 men and 511 women in the study but only 1 malformation in a child born to a female worker exposed during pregnancy and 11 malformation were lower than expected for the national population but the number of children in the study was small and therefore no firm conclusions can be drawn.

An odds ratio of 0.8 was obtained for adverse outcome of pregnancy with styrene exposure in case control studies in Sweden and Norway (Ahlborg *et al,* 1987). Female workers in the polystyrene plastics industry were studied and cases of still births, particular malformations and low birth weight pooled. Again, the number of cases was relatively small (43 cases in Sweden, 10 cases in Norway) and the authors indicate that the study had low power.

Overall, the available studies on congenital malformations have low power but do not show any evidence that styrene has produced such effects in humans.

Other studies

Birth weights were analysed in a US study of mothers who worked when pregnant in the reinforced plastics industry (Lemasters *et al*, 1989). A NIOSH questionnaire was used by professional telephone interview staff to obtain information on reproductive and job histories. Data were collected regarding general consumed by the mother. The study covered 1050 exposed women employed at 36 companies between 1974 and 1981. Controls (485) were selected from office workers and staff from other local companies. The number of unexposed births was increased by including pre-employment births from the subsequently exposed women. An “independent sample” of 229 exposed and 819 unexposed live births was created by taking a randomly selected unexposed birth from a worker who was in the

control group, together with the first exposed birth from woman on the study. Occupational hygiene information was obtained from the companies and from state or federal agencies. Visits were made by one of the authors to investigate 28 of the companies. There was some exposure to other organic solvents, as is normal for the GRP industry. Exposure to styrene was categorised into no, low (<30 ppm) and high (>30 ppm). Exposure levels were obtained from a large number of historical samples but details of the type of mean and reference period were not given.

There were 819, 154, and 75 pregnancies in the no, low and high exposure groups. These groups were comparable in age, gravidity and length of education. A lower proportion of women in the high exposure group were from families with income >$15000. Regression analysis was used to take into account many factors influencing pregnancy outcome such as age. There was no statistically significant effect of exposure to styrene on birth weight. For the 50 births in a subgroup working in jobs expected to have the highest styrene exposures (about 50 ppm), mean birth weight was 4% lower than that for unexposed births after adjustment for other factors but this finding was not statistically significant. Overall, no significant styrene-related effects were observed.

Summary of studies in humans

A range of epidemiological studies, particularly focussing on developmental effects, have been conducted but most of these lacked adequate exposure information and were too small to be conclusive. Nevertheless, the studies have been generally negative and the available human data certainly provides no reliable evidence for styrene exposure-related increases in spontaneous abortions, congenital abnormalities, birth weight, menstrual disorders, fecundity, male or female fertility or sperm quality within the exposure ranges investigated. Overall, there is no clear evidence of an effect of styrene on human reproduction, but data are too limited to exclude the possibility for effects.

There is suggestive evidence that exposure to styrene in occupational settings is associated with increased levels of serum prolactin relative to unexposed individuals (see repeated dose toxicity section, human data, biochemical studies related to nervous system functioning). The interpretation of the clinical relevance of these effects is uncertain because the average elevation was not outside the normal range and because menstrual function and other reproductive endpoints were not evaluated in these studies. Therefore, overall, there is no clear evidence of an adverse effect of styrene on human endocrine function.

4.1.2.9.4 Summary of effects on reproduction

A range of epidemiological studies, particularly focussing on developmental effects, have been conducted but most of these lacked adequate exposure information and were too small to be conclusive. Nevertheless, the studies have been generally negative and the available human data certainly provides no reliable evidence for styrene exposure-related adverse effects in relation to spontaneous abortions, congenital abnormalities, birth weight, menstrual disorders, male fertility or sperm quality within the exposure ranges investigated. Overall, there is no clear evidence of an effect of styrene on human reproduction, but data are too limited to exclude the possibility for effects.

There is suggestive evidence that exposure to styrene in occupational settings is associated with increased levels of serum prolactin relative to unexposed individuals. The interpretation of the clinical relevance of these effects is uncertain because the average elevation was not outside the normal range and because menstrual function and other reproductive endpoints were not evaluated in these studies. Therefore, overall, there is no clear evidence of an adverse effect of styrene on human endocrine function.

A well-conducted two-generation inhalation study found no effects on fertility and reproductive performance in rats exposed to up to 500 ppm (2165 mg/m^3 ≈ 300 mg/kg/day) styrene, a concentration causing parental toxicity (degeneration of the olfactory epithelium and reductions in body weights).

From the other relevant studies available, there is no convincing evidence that styrene can impair reproductive performance, produce testicular toxicity sperm abnormalities or adversely affect the reproductive organs. Thus, taken together, the data available indicate that styrene does not have the potential to impair fertility and reproductive performance in animals.

Data from inhalation and oral developmental toxicity studies in a number of species are available, but most are either poorly designed or reported. There are no studies using the dermal route of exposure.

In the rat, inhalation exposure produced no evidence of significant effects on conventional parameters assessed in the foetus at non-maternally toxic exposure concentrations of up to 600 ppm styrene. However, developmental delays have been reported postnatally in a number of non-GLP and non-OECD studies at 300 ppm styrene in the absence of overt maternal toxicity. This has been confirmed by a recent, well conducted two-generation study in which, a pattern of developmental delay (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength), including decreased body weights, was evident mainly in the F_2 pups of the high exposure group (500 ppm). It is noted that, in contrast to previous investigations, in this OECD- and GLP-compliant study the delay in pup development was seen at an exposure level causing some maternal toxicity (reductions in body weights of 6-7% and degeneration of the nasal olfactory epithelium). No specific developmental neurotoxicity was seen in this study up to the highest tested concentration of 500 ppm.

No convincing evidence of specific developmental effects was seen in other species (mice, rabbits and hamsters) exposed to styrene via the inhalation route of exposure.

Only studies in the rat are available using the oral route of exposure; generally, no significant effects on any of the conventional parameters assessed in the foetus were seen at dose levels up to 250 mg/kg/day, at which maternal toxicity was observed.

Overall, it can be concluded that styrene does not cause developmental toxicity in animals as evaluated by structural endpoints at inhalation exposures of up to 600 ppm and oral exposures of up to 250 mg/kg/day and by neurological endpoints at inhalation exposures of up to 500 ppm. However, reduced pup growth and pup developmental delays (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and small reductions in forelimb grip strength) were seen postnatally in rats at exposure levels (300-500 ppm) causing, in some cases, maternal toxicity. With the exception of a small reduction (up to 10%) in pup body weight, no developmental effects were observed at 150 ppm (650 mg/m^3 ≈120 mg/kg/day) in a well-conducted 2-gen study.Taking into account all of the available information, it is suggested that 150 ppm (650 mg/m^3) is taken forward to the risk characterisation as the NOAEC for potential effects of styrene on development. Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. This information will be taken into account when judging the adequacy of the Margins of Safety in the risk characterisation.

Considerable information from animal and human toxicity data and *in vitro* screening assays, indicate that styrene does not possess any oestrogen-like or androgen-like activity.

4.1.3 Risk Characterisation

4.1.3.1 General aspects

Toxicokinetics - A substantial amount of information is available on the toxicokinetics of styrene in humans, following exposure by the inhalation route; information on percutaneous absorption in humans is also available.

In humans, inhaled styrene vapour (at concentrations of 10-200 ppm) is well absorbed across the respiratory tract. Thus, a value of 100% for absorption via the inhalation route of exposure is taken forward to the risk characterisation.

Dermal absorption of the liquid has been estimated to be approximately 2% of the applied dose in an *in vitro* study using human skin samples. This value is taken forward to the risk characterisation. Dermal uptake of the vapour appears to make only a small contribution (5% or less) to the total body burden arising from combined inhalation and dermal exposure to the vapour.

No information is available on oral absorption in humans, but from the physicochemical properties of styrene and experimental animal information, one would expect extensive absorption from the gastrointestinal tract. Thus, a value of 100% for oral absorption is taken forward to the risk characterisation. Following absorption, it can be predicted from experimental animal data that styrene is widely distributed in humans, and needle biopsy investigations have shown that styrene certainly locates in adipose tissue; there was a correlation between the amount of body fat and the total body burden of styrene. Data on styrene blood levels in human volunteers following single inhalation exposures and in rats exposed via inhalation show that at identical exposure concentrations, styrene blood levels are very similar (e.g. 2.5 and 3.5 μg/ml in rats and humans respectively at 100 ppm styrene).

The rate of absorption following inhalation is much higher (2-3 fold) in mice than in rats. The absorption rate in humans is approximately the same as in rats. The rate of styrene uptake in the upper respiratory tract is partly dependent on its metabolism, and was decreased when animals were pre-treated with a P450 inhibitor.

In humans, styrene is eliminated from the body relatively rapidly, primarily in the urine. However, there is some evidence for modest biopersistence in human adipose tissue on repeated daily exposure. Styrene clearance from blood is biphasic. Half-lives for inhaled styrene were reported at 0.6 hours for the first elimination phase and 13 hours for the second elimination phase. From studies in mice, there is evidence that styrene is also rapidly eliminated from blood following either single or repeated inhalation exposure. A study in pregnant mice has shown that styrene and/or its metabolites can cross the placenta into the foetus.

The metabolism of styrene has been studied thoroughly in mice, rats and humans. A number of metabolic pathways have been identified. The evidence suggests that these pathways are active in mice, rats and humans, although there are species differences in their relative importance.

Styrene is metabolised extensively in all species. According to a PBPK model developed by Ramsey and Andersen, saturation of styrene metabolism in humans occurs at blood levels exceeding 1.7 μg/ml styrene or 200 ppm styrene in air. Below these concentrations, the rate of styrene metabolism is limited by the rate of blood perfusion in the liver or other organs involved in styrene elimination. The first step in the metabolism of styrene involves oxidation of the aromatic ring or side-chain. The main route in each species is the oxidation of the side chain to give the epoxide, styrene-7,8-oxide (SO). A number of studies have demonstrated the involvement of P450 in this step and provided information on the specific P450 isoforms

involved in the production of SO (CYP2E1, CYP2B6 and CYP2C8 in the liver and CYP2F2 and CYP2E1 in the lung). For the lung, the evidence shows that CYP2F2 is the most active isoform involved in the bioactivation of styrene in this tissue, with CYP2E1 playing a less important role. The SO produced is enantiomeric and is produced in the R- and S-forms, probably as a result of metabolism by different P450 isoforms. Different ratios of R-SO to S-SO are found in different tissues and different species. Mouse Clara cells produce about 3 times more of the R-enantiomer than the S-enantiomer, while rat produces more of the S-enantiomer, and humans, like rats, produce more of the S-form. SO is either metabolised further by conjugation with glutathione to give mercapturic acids, or is hydrolysed by epoxide hydrolase (EH) to phenylglycol. This is subsequently metabolised to mandelic, phenylglyoxylic and hippuric acids. P450 and EH are both microsomal enzymes in the endoplasmic reticulum. Therefore, SO produced *in situ* by P450 may potentially be rapidly detoxified if there is sufficient EH present.

Other metabolic pathways can lead to phenylacetaldehyde (PA) and phenylacetic acid (PAA) (via side-chain β-oxidation and hydroxylation), to phenylethanol and acetophenone (via side-chain α-oxidation and hydroxylation), oxidation of the aromatic ring to give 4-vinylphenol (4-VP), and products of ring opening. These metabolites are excreted in the urine. There are studies which have demonstrated that P450 enzymes are also involved in both the side-chain and ring oxidation of styrene and that 4-VP is further metabolised in lung microsomes by specific P450 isoforms to extremely reactive downstream products (e.g. an epoxide and a hydroquinone derivative). Subsequently these derivatives are conjugated with glutathione, but at present there is no information on the relative rates of 4-VP metabolites detoxification between different species.

It is clear that metabolism involving SO as an intermediate is a major route in rodents and humans. However, there are some notable species differences. In humans, almost all of styrene (95%) is metabolised to SO and further metabolised by EH; approximately 5% of styrene is metabolised via the phenylacetaldehyde pathway. No more than trace amounts (<1%) of SO-GSH conjugates or ring-oxidized metabolites of styrene (4-VP) occur in humans exposed to styrene. Further metabolism of SO by EH is important but less extensive in rodents than in humans (68-72% in rats and 49-59% in mice). In rodents, conjugation of SO with GSH is an important route accounting for up to a third of the SO removal. The most significant difference between mice and rats is in relation to the production of phenylacetaldehyde (12-22% in mice against 3-5% in rats) and products of ring-oxidation (4-VP; 4-8% in mice against <1% in rats).

These data indicate significant differences in the metabolism of styrene between species and between tissues. It should be noted that, although these data arise from *in vitro* studies and PBPK modelling, they clearly mirror the toxicodynamic picture of styrene obtained *in vivo*. The tissue specific metabolism of styrene suggests that *in situ* metabolism within each tissue may be a more important determinant of toxicity than the overall systemic metabolism and blood levels of styrene metabolites. The implication of this is that the specifics of the local metabolism in a target tissue must be considered when extrapolating findings in animals to assess the likely hazards and risks in the equivalent human tissues.

Overall, human tissues – apart from the liver - produce very little SO, if any, and have a greater capacity to hydrolyse SO with EH than rodents. This difference is most pronounced in human nasal and lung tissues where production of SO is minimal or undetectable, and is also associated with a greater capacity to hydrolyse SO by EH. The mouse lung and nasal tissues produce the greatest amount of SO among the species tested, and, in general, have less EH activity, suggesting that significantly high local concentrations of SO will be present in these tissues. It is also evident that other toxic metabolites, particularly 4-VP and its reactive downstream products, are produced to a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse concentrations). Although it cannot be ascertained whether or not these species differences in the formation of 4-VP metabolites in the lung may be a reflection of the different numbers of Clara cells

(the metabolically active lung cells) present in the different species, since 4-VP metabolites are produced by the same cytochrome P450 enzymes involved in the production of SO, it is most likely that the species differences in the formation of 4-VP metabolites observed reflect species differences in metabolic capability.

Acute toxicity - In humans, there is some acute inhalation toxicity information available indicating effects of styrene on the central nervous system (CNS) function. From the studies that have been reported there has been no convincing evidence of an effect on neurobehavioural test performance with exposures in the range 0.5 – 150 ppm; however, some impairment in test performance (reaction time, manual dexterity, coordination) appeared with exposures of 200, 350 and 376 ppm for periods of 30 – 90 minutes. Higher concentrations (800 ppm in one study) have produced signs and symptoms of pronounced CNS depression. No other acute toxicity information is available from human studies.

In rats and guinea pigs styrene is of moderate-low acute toxicity via the inhalation and oral routes of exposure. Two inhalation studies in rats have reported a 4-hour LC_{50} of 2770 ppm (11.8 mg/l) and "some deaths" at 2149 ppm (9.1mg/l) for up to 40 hours. An oral LD_{50} of approximately 5000 mg/kg has been reported in rats. In contrast, mice (at least some strains of mice) are much more sensitive to a single exposure to styrene, with cellular damage in the respiratory epithelium at 40 ppm (the lowest concentration tested) and fatal hepatocellular damage at 250 ppm and above in acute inhalation studies. The most likely explanation for this species difference is the greater potential for build-up of the reactive styrene oxide metabolite in the mouse, compared to the rat or humans. An acute oral study in hamsters also indicates styrene-induced hepatotoxicity at 600 mg/kg (but not at 450 mg/kg). No acute dermal toxicity studies have been performed in experimental animals, but one would predict low acute toxicity, with the possible exception of some strains of mouse. In view of the fact that humans (volunteers and workers) have been exposed without serious effects to acute exposure conditions that have proved toxic and even lethal to the more sensitive mouse strains, and considering the known toxicokinetic differences between the mouse and the human in the activation/deactivation of styrene, the mouse is considered to be a poor and unreliable model for the acute toxicity of styrene in humans. Therefore, for the purposes of risk characterization, information from the human volunteer studies will be used. The most useful reference point in relation to short-term single exposure is the observation that no CNS depression was seen in humans exposed to 100 ppm (433 mg/m^3) for 7 hours and that some minor impairment in neurobehavioural test performance was observed at about 200 ppm (866 mg/m^3) and above for 1 hour.

Irritation - Although limited, the available data suggest that liquid styrene is not significantly irritating to the skin after a single exposure, but that repeated exposure does cause irritation. The available evidence suggests that liquid styrene can produce eye irritation. Exposure to airborne styrene vapour can also cause eye irritation. Exposures of up to approximately 216 ppm for 1 hour were without effect but concentrations of 375 ppm and above for 1 hour were clearly irritating. Exposure to 100 ppm for 7 hours caused mild and transient irritation; however, since no effects were seen at 216 ppm for 1 hour, it is considered that the symptoms reported at 100 ppm with a longer exposure are likely to be due to eye dryness or a similar sensation rather than to primary irritation. The NOAEC values of 216 ppm (935 mg/m^3) for 1-hour and of 100 ppm (433 mg/m^3) for 7 hours will be used in the risk characterisation for eye irritation caused by exposure to the vapour. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 100 ppm for 7 hours was not a clear NOAEC.

It is clear that exposures to airborne styrene can cause respiratory tract (nasal) irritation. No effects were seen at 100 ppm for 1 hour or 7 hours and only one subject out of nine reported nasal irritation at 216 ppm, suggesting little or no significant irritation at this concentration. Nasal irritation was more evident at 375 ppm and above, in several studies. For the purpose of risk characterisation, the NOAEC values of 216 ppm (935 mg/m^3) for 1 hour and of 100

ppm (433 mg/m^3) for 7 hours will be used. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 216 ppm for 1 hour was not a clear NOAEC.

Sensitisation - The reporting of the available animal skin sensitisation data is inadequate, precluding a clear conclusion being drawn from the studies themselves. However, given that widespread exposure to styrene has led to only one reported possible case of skin sensitisation, this extensive human experience indicates that styrene is not a significant skin sensitiser and negates the need for any further animal testing with respect to this endpoint. Similarly, there has been extensive inhalation exposure in humans, which has resulted in only two case reports of asthma, each of which has unconvincing aspects to it. This suggests that styrene has no significant asthmagenic potential.

Repeated dose toxicity - There is a large amount of information from studies in humans. In the few studies that have reported on patterns of mortality from non-malignant disease in occupational groups repeatedly exposed to styrene, the authors of the studies have signalled findings that have been proposed to be worthy of further exploration. However, a critical appraisal of the studies, taken together, suggests that they present no convincing evidence that styrene exposure has enhanced the incidence of mortality from any particular disease.

In the available worker health survey studies, consistent evidence of an increase in the self-reporting of symptoms of eye and nasal irritation and CNS disturbance (drowsiness, headache, lightheadedness) comes through. Unfortunately, the quality of the exposure data means that it is not possible to relate these effects to reliable levels of styrene exposure, particularly as it is possible that these effects are related more to short-term peaks in exposure rather than to workshift averages. No reliable evidence for any other effects of styrene is furnished by these studies. Overall, from the available studies there is no convincing evidence of a clear, interpretable and toxicologically significant effect of styrene having occurred in exposed workers, in relation to haematological, immunological, hormonal or renal endpoints.

Because styrene is highly lipid-soluble and, like many other organic solvents, at certain concentrations, produces acute CNS effects, concern about the long-term toxicity of styrene has been focused on its potential for damaging the nervous system. To that end, for the last 40-45 years, several studies investigating the potential neurotoxicity of styrene in exposed workers have been undertaken in factories in many parts of the world. The majority of these studies have suggested that styrene has substantive effects on the nervous system in humans such that the generally uncritical recitation of the results from these investigations has created over the years the label that styrene is a potent neurotoxicant. However, despite this extensive investigation of styrene potential neurotoxicity, a critical review of the available data has shown that there is no clear relationship between repeated exposure to styrene and persisting damage to the nervous system.

Several studies have been conducted on EEG patterns in styrene-exposed workers. Overall, the collective findings do not provide robust evidence for the absence or presence of styrene-induced EEG changes in exposed workers. Furthermore, although the approach is valuable in that it provides measures of nervous system function that are independent of the level of collaboration of the subject, no clear criteria for interpretation of the health significance of any EEG changes that might have occurred are available. If styrene was the causal agent of EEG changes in workers covered by these studies, the most likely interpretation is that the effects were due to the general CNS depressant action of styrene.

The available nerve conduction studies have produced inconsistent results for different groups of workers exposed to similar levels of styrene. Overall, it is not clear if styrene exposure can produce a decrease in nerve conduction velocity; furthermore, if it can, the underlying basis for the effect could not be deduced from the available information. Also, the

clinical significance of this effect is questionable as all subjects appeared to be healthy workers.

A few studies are available which were designed to investigate any effect of styrene on hearing function. When confounding factors such as age and noise exposure were taken into account, no relationship between styrene exposure and hearing loss was found. There is limited evidence that styrene exposure may have caused minor effects on vestibular reflexes in some workers. However, the quality of the exposure data is such that it is not possible to relate these effects to reliable levels of styrene exposure. Therefore, although these human data cannot be used for risk characterisation purposes, nevertheless they indicate that the observations of ototoxicity in animals may be relevant to humans.

In one study to investigate olfactory function, other than an adaptive increase in the threshold for odour detection of styrene itself, no olfactory deficit was evident in workers exposed to up to 77 ppm (8-hour TWA).

Studies on colour vision provide some evidence to support the view that styrene does cause changes in colour discrimination relative to age-matched controls. However, it should be noted that, as a single colour vision test rather than a testing battery approach was used, these findings are not sufficiently robust to reliably characterise the scale and the nature of the effect. Generally, the effect was on the tritan (blue-yellow) type, although some workers also had evidence of red-green colour vision deficiency. However, there is a lack of information on whether the effect is related to short-term or repeated long-term exposures to styrene. There is also a lack of information on the magnitude of the effect in workers exposed to styrene concentrations > 100 ppm (8h TWA), although performance in colour discrimination tests at these high exposures is likely to be subject to confounding because of transient CNS depression or eye irritation. Studies suggest that changes in colour discrimination would not be expected with 8 h TWA exposures below 20 ppm. Therefore, although there are findings suggesting minor changes in colour discrimination at relatively high levels of exposure to styrene ($\geq$ 50 ppm, 8h TWA), it should be noted that the available studies are not sufficiently robust to reliably characterise the scale and the nature of the effect. Also, given the very mild nature (the affected workers were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, it is deemed that the slight changes in colour discrimination detected should not be considered as an adverse health outcome of styrene exposure. It can be concluded that since the effects observed at 50 ppm (216.5 mg/m^3; 8h TWA) are not yet adverse, this exposure value can be considered a NOAEC against which risk characterisation should be performed.

Numerous workplace studies using neurobehavioural testing are available. The results obtained have been variable, with some studies reporting effects and others no effects for workers within similar exposure ranges. Performance was adversely affected in several studies in only a small proportion (1-3) of the tests administered (6-20). Also, different types of neurobehavioural test (representing various functional domains of the CNS) were apparently sensitive to styrene exposure in different studies. This lack of a clearly consistent effect on particular functional domains indicates that there is only weak evidence for a causal relationship. In some studies, there is a possibility that effects similar to those detected in volunteer studies (i.e. slightly slower response times) were observed and these might be related to brief peaks of styrene exposure of hundreds of ppm that had occurred during the previous shift. In view of this, it is not possible to discern a clear dose-response relationship for any of the effects observed in neurobehavioural tests in workers. Furthermore, at present there is not a clearly established, widely accepted interpretational framework into which these results can be fitted. Where apparent deficits in neurobehavioural test performance have been measured, the underlying toxicological processes involved, the consequences of the health and safety of the individual, and the effect of styrene in comparison with that of other experiences and phenomena regularly encountered in everyday life, have not been established.

Taking all of these points into consideration, the rapporteur proposes that the crucial issue in relation to the impact of styrene on the nervous system is the need to avoid acute CNS depressant effects and associated symptomatology.

In relation to findings in experimental animals, a variety of repeated inhalation exposure studies in different animal species are available. However, among these species, the rat and mouse have been the most extensively investigated. Four well characterised target sites of toxicity have been identified: the nasal epithelium (in rats and mice), the lung (in the mouse), the liver (in the mouse) and the ear (in the rat).

In relation to nasal epithelium damage, a NOAEC has not been identified, with chronic inflammatory changes (mild in degree and confined to the olfactory epithelium with no effects on Bowman's glands or olfactory nerve fibres), having been seen in the rat with long term daily exposure to 50 ppm styrene, the lowest concentration explored in a 2-year study. Nasal toxicity has also been reported in mice. In a similar 2-year study, respiratory metaplasia of the olfactory epithelium, hyperplasia and hypertrophy of the Bowman's gland and atrophy of the olfactory nerve fibres were observed starting from 20 ppm, the lowest concentration tested. It is clear that the nasal lesions induced by styrene exposure are a lot more severe in the mouse compared to the rat. Over the years a number of investigative studies have been undertaken to characterise and explain these species differences and to investigate the relevance of these findings to humans. The results of these investigations have shown that the differences in nasal toxicity between rat and mouse can be explained by the greater ability of the rat nasal epithelium to detoxify reactive metabolites of styrene formed *via* CYP2F2 metabolism. These reactive/toxic intermediates include styrene oxide and most probably the downstream metabolites of 4-VP. Detoxification of toxic species by epoxide hydrolase is 10-fold higher in the rat olfactory tissue as compared to the mouse while glutathione S-transferase activity is approximately 3-fold higher in the rat nasal tissue as compared to the mouse. It is important to consider that cytochrome P450-catalysed metabolic activation of styrene to styrene oxide in human nasal epithelial tissue appears to be negligible (see toxicokinetics section). Also, since uptake of styrene by nasal tissue is enhanced by the ability of the tissue to metabolize styrene, the lack of the primary step of styrene metabolism in human nasal tissue serves as a further reason for dismissing the relevance to humans of these rodent nasal lesions. Besides these metabolic differences, anatomically, there are significant differences in the nasal passages of rodents and humans, which result in significant different volumes and airflow patterns. Thus, although inhaled styrene may be deposited in the nasal passages of humans, it is likely that high levels will not be deposited in the olfactory area. Furthermore, human investigations have shown that exposure up to 77 ppm (8h TWA) styrene as occurring in the UP-resin industry is not associated with impairment of olfactory function, and in several human health surveys of workers repeatedly exposed to styrene up to a maximum of approximately 700 ppm, no nasal lesions have been described (see RDT, human studies section). Hence, it can be concluded that rodent nasal epithelium damage induced by styrene is not of relevance in relation to the potential repeated dose effects of styrene in humans at relevant levels of exposure.

In relation to the lung, no effects were seen in rats exposed up to 1000 ppm, but in mice a NOAEC was not identified, with damage to the lung epithelium (hyperplasia in the terminal bronchioles, focal bronchiolar-alveolar hyperplasia, fibrosis extending to the alveolar ducts and extension of non-ciliated cells from the terminal bronchiole to the alveolar duct) having been seen from 20 ppm, the lowest exposure concentration tested in a 2-year study. Work with the cytochrome P450 inhibitor 5-P-1-P has shown that metabolic activation of styrene to styrene oxide and other reactive metabolites (e.g the downstream metabolites of 4-vinylphenol) and the subsequent detoxification of styrene oxide are crucial elements of this toxic response. This is supported by the observation that it is the metabolically active Clara cells that are the initial focus of damage. These non-ciliated bronchiolar epithelial cells are mainly involved in the metabolism of xenobiotics, but also in the secretion of surfactants and

in the renewal process of the bronchiolar epithelium (Komaromy and Tigyi, 1988). Early biochemical changes, sustained cell damage and regenerative cell proliferation were observed in lung Clara cells of mice exposed to 40 and 160 ppm styrene for up to 4 weeks. In this context it is important to consider that cytochrome P450-catalysed metabolic activation of styrene in human lung tissue appears to be minimal (see toxicokinetic section) even though the two main P450 isoforms involved in styrene metabolsim, CYP2E1 and CYP2F1, have been detected; that the number of Clara cells in human lung is very low; and that their most important functions in human lung are shared by other cell types (e.g. Type II cells). Hence, it can be concluded that these lung tissue findings in mice reflect a toxic response that will not occur to any significant extent in humans at relevant levels of exposure.

In relation to the liver, exposures in the range 150-350 ppm have produced fatal hepatotoxicity in mice. Again, it appears that the metabolic activation of styrene to SO is a crucial stage in the hepatotoxicity process. In this context, it is evident that the toxicokinetic information available suggests that the mouse would be more susceptible than humans to styrene induced hepatotoxicity. This is borne out by the observation of mouse fatalities at exposure levels to which humans have been regularly exposed in the past, with no reports of hepatotoxicity or death.

In relation to effects on the ear, clear evidence of ototoxicity (both functional and histological has been seen in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In three different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter two cases the studies were only of four weeks' duration. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tend to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity of the animals. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm. Comparative studies using rats and guinea pigs exposed to 1000 ppm for 5 days indicate an obvious species-difference, as similar findings were not observed in guinea pigs. Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. The underlying toxicological mechanism has not been clearly elucidated. This effect should be therefore regarded as of potential relevance to human health. The histological damage consists of the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irriversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organisation of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. On this basis, it is considered that the NOAEC values of 500 and 300 ppm for 4 weeks in sedentary/ordinary and active rats respectively are a more accurate starting point for risk characterisation than the NOAEC of 200 ppm for 13 weeks.

In one single non-standard investigation, rather minor effects on the number of the large amacrine cells and on the content of neuramines and glutathione of the retina of rats exposed repeatedly to 300 ppm styrene for 12 weeks have been reported. However, it is considered that, in the absence of any associated conventional histopathology and given the lack of information on what are the 'normal' levels of dopamine and glutathione in the retinal cells of rats, the rather minor morphometric and biochemical changes observed are unlikely to represent a toxic response.

Overall, the available animal inhalation repeated dose toxicity studies have identified ototoxicity as the most sensitive and relevant effect of styrene repeated inhalation exposure with NOAEC values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) for 4 weeks in sedentary/ordinary and active rats respectively.

Most of the available repeated oral exposure studies have been performed in rats and mice. Information from a carcinogenicity bioassay in the rat has shown no evidence of treatment related toxicity in animals administered 1000 mg/kg/day for two years; a marked increase in mortality was evident at 2000 mg/kg/day. In another 2-year study, styrene did not produce any clear evidence of toxicity when administered in drinking water at a dose of 21 mg/kg/day, the highest dose level tested. However, it is noted that potential effects on the ear were not investigated in these studies. Ototoxicity, a clearly established effect of styrene in the rat, was seen in one study at 800 mg/kg/day (and perhaps, although less convincingly, at 400 mg/kg/day) for 2 weeks.

In mice, the most reliable information comes from a cancer bioassay, in which increased mortality and hepatic necrosis were observed at the highest dose of 300 mg/kg/day; a NOAEL of 150 mg/kg/day was identified from this study. The one significant observation from the remaining studies is that of toxicity towards the lung epithelium, adding further support to the concept that the lung toxicity of styrene in mice, following oral and inhalation exposure, results from local metabolism of styrene to styrene oxide and to other reactive metabolites (e.g. the downstream metabolites of 4-vinylphenol).

Overall, in relation to repeated oral exposure, the NOAEL of 150 mg/kg/day identified from a 2-year cancer bioassay in the mouse should also be considered. But in extrapolation to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat.

No repeated dermal studies are available, although low systemic toxicity would be predicted in most conventional experimental species with the possible exception of some strains of the mouse.

Mutagenicity - With respect to mutagenicity, a large number of studies have been published which have aimed to investigate the genotoxic potential of styrene in humans by examination of various endpoints in styrene exposed workers. Very low levels of DNA adducts were found in some styrene exposed workers but it has been stated that such low levels should be viewed with caution. There is also some evidence of DNA damage (SSBs) induced in styrene exposed workers. Both these endpoints are indicative of exposure and are not necessarily associated with heritable effects. The results of several studies on another indicator endpoint of unclear health significance, SCEs, did not provide evidence of a positive response, despite these being induced in animals exposed to styrene. There are also many studies investigating endpoints (gene mutations, chromosome aberrations and micronuclei) known to lead to heritable effects. The number of studies assessing gene mutation is very limited and no conclusions can be drawn from them. Although 5 studies appear to present evidence that styrene may be weakly clastogenic in humans, there are 11 robust negative studies also. Together with a lack of evidence of a dose-response relationship and the negative response for induction of micronuclei when studied concurrently in two of the positive chromosome aberration studies, no clear conclusion on *in vivo* clastogenicity of styrene in humans can be made.

Overall, given the lack of evidence of consistent relationships between exposure levels and study outcome, the lack of any consistent profile of endpoints and the absence of information on the relevance of the types of adducts seen and their mutagenic potential *in vivo*, there is no convincing evidence that styrene has shown mutagenic activity in humans.

The overall picture presented by the *in vitro* assay results available is that at least in some test systems (including Ames tests and *in vitro* chromosome aberration studies in

mammalian cells), styrene does possess some genotoxic potential *in vitro*. Metabolic activation (presumably to styrene oxide) is required for this activity. Styrene has been exhaustively studied in clastogenicity studies in animals up to concentration and/or dose levels producing severe toxicity in some cases. There is no convincing evidence of styrene clastogenicity when the quality of the studies and the plausibility of the test results are considered. Equivocal results were obtained after exposure to high concentrations and/or doses causing lethality. However, overall, negative results were obtained from *in vivo* chromosome aberration and micronucleus studies in the rat, hamster and the mouse following single or repeated exposures to styrene up to concentrations and/or doses causing systemic toxicity, via the inhalation, oral and intraperitoneal route in the tissues examined (bone marrow, peripheral lymphocytes, splenocytes and whole blood). Furthermore, a recently published micronucleus test in bone marrow cells of mice conforming to the current OECD guideline was clearly negative.

The general pattern of SCE results in the wide range of tissues examined (lymphocytes, spleenocytes, bone marrow, alveolar macrophages, regenerating liver cells) from both the rat and the mouse following inhalation or i.p exposure to styrene has been positive. However, it is important to note that in most cases concomitant chromosome aberration and/or micronucleus assays involving the same animals and in some cases the same tissues were carried out and that negative results were obtained for these indicators of chromosome damage. Therefore, this clearly reduces the significance of these SCE findings in relation to mutagenicity.

The binding of styrene metabolites to DNA was very low and did not indicate any specificity for the target tissue (mouse lung). Induction of alkali-labile single-strand breaks has also been produced *in vivo* in rats and mice exposed to styrene. Again the significance of these findings is unclear, given the repeated failure of styrene to demonstrate mutagenic activity in standard clastogenicity assays.

In summary, the available data suggest that styrene is weakly positive in indicator tests detecting SCEs, DNA stand breaks and DNA adducts. In contrast, an in vivo UDS test performed in accordance with international guidelines did not reveal a genotoxic effect of styrene in mouse liver.

Overall, based on standard regulatory tests, there is no convincing evidence that styrene possesses significant mutagenic/clastogenic potential *in vivo* from the available data in experimental animals.

Carcinogenicity - In relation to carcinogenicity, several cohort and case-control studies covering workers exposed to styrene in the GRP and styrene production industries are available. In these studies there was no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene. The increased risks for lymphatic and haematopoietic neoplasms observed in some of these studies were generally small, statistically unstable and often based on subgroup analyses. These findings are not very robust and the possibility that the observations are the results of chance, bias or confounding by other occupational exposures cannot be ruled out. In the styrene-butadiene rubber industry, several studies have pointed to an increased risk of cancer of the lymphatic and haematopoietic systems. However, detailed analysis of these data, together with the general toxicological picture for styrene and butadiene, suggests that where increases are due to occupational exposure, it is butadiene, not styrene, that is the more likely causative agent. In conclusion, based on human studies, there is no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene.

In animals, the carcinogenic potential of styrene has been explored in rats and mice, using the inhalation and oral routes of exposure. A carcinogenic effect of styrene towards the lung is evident in the mouse. This has been shown in a well-conducted lifetime inhalation study in CD1 mice at exposure concentrations of $\geq$ 20 ppm styrene and, somewhat less convincingly, in an oral study in mice of the $B6C3F_1$ strain. The inhalation study, which included extensive

histopathological examination, showed that the tumours (prevalently adenomas) were preceded by cytotoxicity characterised by early Clara cell toxicity followed by progressive bronchiolar epithelial hyperplasia and bronchiolar-alveolar hyperplasia.

In the rat, styrene has not exhibited any clear evidence of carcinogenic potential by the inhalation or oral route. In individual studies there have been isolated findings of statistically significantly higher incidences of various particular tumour types in particular groups of styrene-treated animals, compared with the in-study controls. However, the findings have been within historical background ranges, not reproducible between studies, in some cases have not shown an upward trend with increasing dose, and have not been associated with evidence of underlying styrene-induced changes at the site in question.

On the question of the relevance of the mouse lung tumours for human health, consideration of the available toxicokinetic information and data from single and repeated inhalation exposure studies in experimental rodents suggests the following as the most plausible toxicological mechanism for these tumours. Styrene is metabolised by cytochrome P450 enzymes in the metabolically active Clara cells (non-ciliated bronchiolar epithelial cells involved in the metabolism of xenobiotics, but also in the secretion of surfactants and in the renewal process of the bronchiolar epithelium) of the bronchiolar epithelium of the mouse, producing cytotoxic metabolites of styrene including styrene 7,8 oxide (SO) and oxidative metabolites of 4-vinylphenol (4-VP). These metabolites cause early Clara cell toxicity/death and sustained regenerative bronchiolar cell proliferation which, in turn, leads to compensatory bronchiolar epithelial hyperplasia and ultimately tumour formation. Clara cell toxicity could also be a consequence of the long term depletion of glutathione, because of conjugation with SO. Genotoxicity of SO (an EU-category 2 and IARC group 2A carcinogen) or other reactive styrene metabolites is unlikely to be involved in tumour development as minimal binding of styrene metabolites to DNA has been detected in mouse lung with no species- or tissue-specificity.

All of the key events of this postulated mode of action are less operative in the non-responsive rat (which does not develop lung tumours at exposure concentrations up tp 1000 ppm) and even less operative in humans.

The number of Clara cells (being responsible for both the formation of toxic metabolites and the target for their toxic action) is very low in humans, even less than in rats. While Clara cells comprise about 85% of bronchiolar epithelium in mice and 25% in rats, in humans such cells are rare.

Although the enzymes CYP2E1 and CYP2F2 required for the formation of the Clara cell toxicants such as SO (including the highly pneumotoxic R-enantiomer) and the downstream metabolites of 4-VP have been detected in human lung, their activities are low (at least 400 times lower than in the mouse) and metabolic activation of styrene to SO is minimal or undetectable. In human lung, detoxification of SO (if formed at all in human pulmonary tissue) takes place predominantly *via* epoxide hydrolase (located on the endoplasmatic reticulum in close proximity to the toxifying cytochrome P450s). The close proximity of the “detoxifying” enzymes to any “toxifying” enzymes ensures the efficient removal of any toxic metabolites. Rodents use both epoxide hydrolase and glutathione-S-transferase as detoxification pathways with the mouse relying on glutathione conjugation more so than the rat. As glutathione S-transferase is located in the cytosol, this makes this detoxification pathway less efficient than the epoxide hydrolase pathway. In comparison to the rodent species, in humans, SO detoxification proceeds nearly exclusively via epoxide hydrolase and glutathione S-transferase accounts for only 0.1% of SO detoxification.

Taking account both of the toxification to SO and its detoxification, PBPK-modelling has shown that the SO content of human lungs is very small, if there is any.

Formation of 4-VP and its downstream metabolites occurs at a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse

concentrations). Although it cannot be ascertained whether or not these species differences in the formation of 4-VP metabolites in the lung may be a reflection of the different numbers of Clara cells (the metabolically active lung cells) present in the different species, since 4-VP metabolites are produced by the same cytochrome P450 enzymes involved in the production of SO, it is most likely that the species differences in the formation of 4-VP metabolites observed reflect species differences in metabolic capability.

As indicated by PBPK-modelling, glutathione depletion caused by SO does not occur in humans. Also, as reactive downstream metabolites of 4-VP are formed in human lung only to a very small extent, the 4-VP metabolic pathway is not expected to cause any glutathione depletion in human pulmonary tissue.

There is no evidence from extensive epidemiological investigations that long term exposure to styrene has produced lung damage or lung cancer in humans.

Hence, overall, the weight of evidence appears to indicate that the consequences of long term exposure to styrene in mouse lung cannot be replicated in the human situation at relevant levels of exposure. Although there are still some uncertainties in this postulated mode of action and in its relevance to humans, namely the lack of data on the relative rates of 4-VP metabolites detoxification in different species, no alternative modes of action that logically present themselves can be supported by as significant a body of evidence as the one presented in this assessment. Consequently, it is felt that the level of confidence in the postulated mode of action can be reasonably high and that, in view of the extensive negative lung epidemiology, it is reasonable to conclude that the lung tumours seen in mice are unlikely to be of any relevance for human health at relevant levels of exposure. A more detailed analysis (according to the IPCS framework for evaluating a mode of action in chemical carcinogenesis) of the evidence in support of the proposed mode of action and of its relevance for human health is presented in Annex A to this document. The carcinogenicity of styrene was evaluated by IARC in 2002. Styrene was considered *possibly carcinogenic to humans* (Group 2B). The Working Group concluded that based on metabolic considerations, it is likely that the proposed mechanism involving metabolism of styrene to styrene 7,8-oxide in mouse Clara cells is not operative in human lungs to a biologically significant extent. However, based on the observations in human workers regarding blood styrene 7,8-oxide, DNA adducts and chromosomal damage, it cannot be excluded that this and other mechanisms are important for other organs.

In the Rapporteur's view, pointing to a possible carcinogenic potential of styrene in other organs is highly speculative as: a) Several large cohort and case-control studies of workers exposed to styrene have shown no evidence for a causative association between styrene exposure and cancer in humans at any site; b) No consistent evidence for styrene-induced toxicity in any organ has emerged from studies of exposed workers; c) The level of DNA damage found in workers exposed to styrene is very low (10-fold lower than that produced by endogenously-generated genotoxic substances such as ethylene oxide) and thus cannot be considered to be of any relevance for subsequent tumour formation. Mechanistic studies have shown that styrene-oxide (SO) and its genotoxicity are not the driving force for lung tumour formation in mice, the only experimental tumour site observed so far. Furthermore, DNA adducts in animals after styrene exposure do not show any specific species or target organ relationship. For example, there is no excess of SO-adduct formation in tissues where SO is formed (e.g. in the liver) at high levels; d) Chromosomal damage caused by styrene exposure in humans is far away from being conclusive. Although 5 studies appear to present evidence that styrene may be weakly clastogenic in humans, there are 11 robust negative studies also. Together with a lack of evidence of a dose-response relationship and the negative response for induction of micronuclei when studied concurrently in two of the positive chromosome aberration studies, no clear conclusion on *in vivo* clastogenicity of styrene in humans can be made. Furthermore, at much higher exposures such effects were not observed in experimental animals.

Reproductive toxicity - A range of epidemiological studies, particularly focusing on developmental effects, have been conducted but most of these lacked adequate exposure information and were too small to be conclusive. Nevertheless, the studies have been generally negative and the available human data certainly provide no reliable evidence for styrene exposure-related adverse effects in relation to spontaneous abortions, congenital abnormalities, birth weight, menstrual disorders, fecundity, male or female fertility or sperm quality within the exposure ranges investigated. Overall, there is no clear evidence of an effect of styrene on human reproduction, but data are too limited to exclude the possibility for effects.

There is suggestive evidence that exposure to styrene in occupational settings is associated with increased levels of serum prolactin relative to unexposed individuals. The interpretation of the clinical relevance of these effects is uncertain because the average elevation was not outside the normal range and because menstrual function and other reproductive endpoints were not evaluated in these studies. Therefore, overall, there is no clear evidence of an adverse effect of styrene on human endocrine function.

A well-conducted two-generation inhalation study found no effects on fertility and reproductive performance in rats exposed to up to 500 ppm (2165 mg/m^3 ≈ 300 mg/kg/day) styrene, a concentration causing parental toxicity (degeneration of the olfactory epithelium and reductions in body weights).

From the other relevant studies available, there is no convincing evidence that styrene can impair reproductive performance, produce testicular toxicity, sperm abnormalities or adversely affect the reproductive organs. Thus, taken together, the data available indicate that styrene does not have the potential to impair fertility and reproductive performance in animals.

Data from inhalation and oral developmental toxicity studies in a number of species are available, but most are either poorly designed or reported. There are no studies using the dermal route of exposure.

In the rat, inhalation exposure produced no evidence of significant effects on conventional parameters assessed in the foetus at non-maternally toxic exposure concentrations of up to 600 ppm styrene. However, developmental delays have been reported postnatally in a number of non-GLP and non-OECD studies at 300 ppm styrene in the absence of overt maternal toxicity. This has been confirmed by a recent, well conducted two-generation study in which, a pattern of developmental delay (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength), including decreased body weights, was evident mainly in the F_2 pups of the high exposure group (500 ppm). It is noted that, in contrast to previous investigations, in this OECD- and GLP-compliant study the delay in pup development was seen at an exposure level causing some maternal toxicity (reductions in body weights of 6-7% and degeneration of the nasal olfactory epithelium). No specific developmental neurotoxicity was seen in this study up to the highest tested concentration of 500 ppm.

No convincing evidence of specific developmental effects was seen in other species (mice, rabbits and hamsters) exposed to styrene via the inhalation route of exposure.

Only studies in the rat are available using the oral route of exposure; generally, no significant effects on any of the conventional parameters assessed in the foetus were seen at dose levels up to 300 mg/kg/day, at which maternal toxicity was observed.

Overall, it can be concluded that styrene does not cause developmental toxicity in animals as evaluated by structural endpoints at inhalation exposures of up to 600 ppm and oral exposures of up to 250 mg/kg/day and by neurological endpoints at inhalation exposures of up to 500 ppm. However, reduced pup growth and pup developmental delays (delays in

attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength) were seen postnatally in rats at exposure levels (300-500 ppm) associated in some cases with maternal toxicity. With the exception of a small reduction (up to 10%) in pup body weight, no developmental effects were observed at 150 ppm (650 mg/m^3 ≈120 mg/kg/day) in a well-conducted 2-gen study.

Taking into account all of the available information, it is suggested that 150 ppm (650 mg/m^3) is taken forward to the risk characterisation as the NOAEC for potential effects of styrene on development. Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. This information will be taken into account when judging the adequacy of the Margins of Safety.

Summary - The hazardous properties of styrene have been evaluated to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of acute toxicity (CNS depression), skin, eye and respiratory tract irritation, repeated dose toxicity and developmental toxicity have been identified. For acute toxicity a NOAEC of 100 ppm (433 mg/m^3) for a 7-hour exposure has been identified in humans for CNS depression based on some minor impairment in neurobehavioural test performance (reactione time, manual dexterity and coordination) at about 200 ppm (866 mg/m^3) and above for 1 hour exposure. No quantitative data are available for skin and eye irritation for liquid styrene, although NOAEC values of 216 ppm (935 mg/m^3) for 1-hour and of 100 ppm (433 mg/m^3) for 7 hours have been identified for eye and respiratory tract irritation from exposure of human volunteers to the vapour. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 100 ppm for 7 hours was not a clear NOAEC for eye irritation as sensations of mild and transient eye dryness were reported at this exposure concentration, and that 216 ppm for 1 hour was not a clear NOAEC for respiratory tract irritation as one out of nine volunteers reported nasal irritation.

For repeated dose toxicity, although there is a large amount of information from studies in humans, a critical review of all the available data has shown that, with the exception of minor changes in colour vision discrimination, there is no clear relationship between repeated exposure to styrene and adverse effects in humans. Changes in colour discrimination have been detected at exposure levels around 50 ppm (216.5 mg/m^3; 8h TWA) and above. However, it should be noted that the available studies are not sufficiently robust to reliably characterise the scale and the nature of the effect. Also, given the very mild nature (the affected workers were not even aware of any deficit) and the likely reversibility of the effect which appears not to affect performance in jobs that require good colour vision, it is deemed that the slight changes in colour discrimination detected should not be considered as an adverse health outcome of styrene exposure. It can thus be concluded that since the effects observed at 50 ppm (216.5 mg/m^3; 8h TWA) are not yet adverse, this exposure value can be considered a NOAEC against which risk characterisation should be performed. In order to compare this human NOAEC with the body burdens arising from exposure to styrene, the selected NOAEC has been converted to an internal NAEL (No Adverse Effect Level) of about 32.6 mg/kg/day by assuming a breathing rate of 1.25 m^3/h, 8 hour exposure, 100% inhalation absorption, 70 kg bodyweight and 5% contribution from dermal absorption of the vapour (216.5x1.25x8/70 = 31 mg/kg/day x 100/95 = 32.6 mg/kg/day). In relation to repeated dose toxicity identified from animal studies, the available inhalation studies have identified ototoxicity as the most sensitive and relevant effect of styrene repeated inhalation exposure with NOAEC values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) in sedentary/ordinary and active rats respectively (equivalent to internal NAEL values of 300 and 360 mg/kg/day in sedentary/ordinary and active rats respectively based on the Sarangapani PBPK modelling; for the active rats it has been assumed that the ventilation

rate is 2-fold faster than that in sedentary/ordinary rats) for 4 weeks. Of these two internal NAEL values, only the lower one (300 mg/kg/day) will be used in the calculation of the MOSs as this is the most conservative approach. Since it is unclear from the data that have investigated this potential effect of styrene in humans whether the human NOAEC of 50 ppm selected for effects on colour vision will also cover for potential ototoxic effects of styrene, the animal NOAEC values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) in sedentary/ordinary and active rats respectively selected for ototoxicity should also be considered in the evaluation of risks posed by repeated exposure to styrene. For repeated oral exposure scenarios, the NOAEL of 150 mg/kg/day identified from a 2-year cancer bioassay in the mouse should also be considered. But in extrapolation of this oral NOAEL to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat.

In relation to developmental toxicity, a NOAEC of 150 ppm (650 mg/m^3 ≈120 mg/kg/day) has been identified in a well-conducted 2-gen study in the rat based upon pup delayed growth (reduced body weights, delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength). Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. This information will be taken into account when judging the adequacy of the Margins of Safety.

There are no concerns for sensitisation, mutagenicity or carcinogenicity. Thus, conclusion (ii) is reached for these endpoints, for all exposure scenarios.

4.1.3.2 Workers

Comparison between exposure and effects

Acute toxicity

In relation to CNS depression, no effects were seen in humans exposed to 100 ppm (433 mg/m^3) for 7 hours, equivalent to an internal 7h-NAEL of about 57 mg/kg (assuming a breathing rate of 1.25 m^3/h, 7 hour exposure, 100% inhalation absorption, 70 kg bodyweight and 5% contribution from dermal absorption of the vapour: 433x1.25x7/70=54.13 mg/kgx100/95=57 mg/kg).

Since the NOAEC for CNS depression was identified following a 7 hours exposure to styrene, the NOAEC will be compared with the RWC 8h-TWA exposure values for the different scenarios rather than with the RWC short-term (15 min) TWA values. To assess the total body burden for workers exposed in the different scenarios, the dose absorbed via the respiratory tract in an 8h-working day has been combined with the dose of the vapour absorbed via the skin and also with the absorbed dermal dose of the liquid. The body burden deriving from absorption of styrene vapour via the respiratory tract for a 70 kg worker has been estimated assuming that 10 m^3 of air is inhaled in a working day and that 100% of the inhaled dose is absorbed. Since dermal absorption of styrene vapour contributes approximately 5% to the total amount absorbed via the respiratory tract and skin combined, the dose absorbed via the respiratory tract represents only 95% of the total amount of the vapour absorbed. This total has therefore been calculated by multiplying the dose absorbed via the respiratory tract by 100/95. The body burden deriving from the RWC dermal exposure to liquid styrene for a 70 kg worker has been estimated by taking into account the predicted exposed surface area for the different scenarios and that only 2% of the dermal

dose is absorbed. The total body burden has then been calculated by adding the two estimates together.

The body burdens arising from the RWC inhalation and dermal exposures in each different worker exposure scenario, and the resultant MOSs derived from comparison with the internal human 7h-NAEL for acute CNS depression are shown in **Table 4.25**.

Table 4.25: Body burdens and MOSs for acute CNS depression

Scenario	RWC 8h-TWA inhalation exposure (mg/m^3)	RWC 8h inhalation body burden including dose of the vapour absorbed via the skin (mg/kg)	RWC 8h dermal exposure to the liquid (mg/day)	RWC 8h dermal body burden (mg/kg)	RWC 8h total body burden (mg/kg)	MOS based on the human 7h-NAEL for acute CNS depression of 57 mg/kg	Conclusion
Manufacture of monomer	4.33	0.65	42	0.012	0.662	86	(ii)
Production of polystyrene	4.33	0.65	42	0.012	0.662	86	(ii)
Production of UP-styrene resin	86.6	13	84	0.024	13.024	4.4	(ii)
Production of SBR and SB latex	21.65	3.26	42	0.012	3.272	17	(ii)
GRP manufacture	433	65	6560	1.87	66.87	**0.9**	**(iii)**

For the majority of the scenarios (manufacture of monomer, production of polystyrene and production of SBR and SB latex), the MOS values are $\geq$ 17. These MOSs are considered to provide sufficient reassurance that CNS depressant effects will not occur, even after allowing for human individual variability in kinetics and dynamics. Therefore conclusion (ii) is reached for these scenarios.

For production of UP-styrene resin the MOS is 4.4. Although this value would not normally provide enough reassurance that CNS depressant effects will not occur after allowing for human individual variability, given that the exposure values are likely to represent overestimates and that only some minor impairment in neurobehavioural test performance was seen at the next exposure concentration level (around 200 ppm), overall, it is reasonable to conclude that the MOS obtained for this scenario is sufficient. Conclusion (ii) is therefore proposed.

For GRP manufacture the MOS is 0.9, which indicates that workers exposed to RWC conditions may be at risk of experiencing CNS depressant effects in this scenario and therefore **conclusion (iii)** is reached. If the typical inhalation (173 mg/m^3) and dermal (984 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 26.28 (26+0.28) mg/kg which is 2.2 below the human NAEL of 57 mg/kg. This MOS does

not provide sufficient reassurance that CNS depressant effects will not occur in this scenario even under typical exposure conditions, and therefore **conclusion (iii)** is also reached for typical exposures.

Irritation

For all scenarios except GRP manufacture, the skin and eye irritation of the liquid substance is unlikely to be expressed during normal handling and use because dermal exposures are low, providing good occupational hygiene practices are in operation. However, if there is contact with the skin or eye (particularly repeated contact in the case of the skin), which could occur accidentally, then local damage is possible. Overall, conclusion (ii) is reached for these scenarios.

For GRP manufacture, it is considered that control of exposure of skin and eyes to liquid styrene in this scenario cannot be anticipated to be adequate and therefore there is a concern that skin and eye irritation from exposure to liquid styrene may be experienced in these industry sectors. Therefore **conclusion (iii)** is reached.

Eye irritation

For exposure to styrene vapour, NOAEC values of 100 ppm (433 mg/m^3) for 7 hours and of 216 ppm (935 mg/m^3) for 1-hour have been identified from exposure of human volunteers. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 100 ppm for 7 hours was not a clear NOAEC for eye irritation as sensations of mild and transient eye dryness were reported at this exposure concentration. A comparison of these NOAEC values with the RWC 8h-TWA and short-term exposures respectively for the different scenarios is shown in **tables 4.26** and **4.27**.

Table 4.26: MOSs for eye irritation (vapour) based upon the RWC 8h-TWA exposures

Process	**RWC 8h-TWA inhalation exposure (mg/m^3)**	**MOS based on the human 7h-NOAEC for eye irritation of 433 mg/m^3**	**Conclusion**
Manufacture of monomer	4.33	100	(ii)
Production of polystyrene	4.33	100	(ii)
Production of UP-styrene resin	86.6	5	(ii)
Production of SBR and SB latex	21.65	20	(ii)
GRP manufacture	433	**1**	**(iii)**

Table 4.26: MOSs for eye irritation (vapour) based upon the RWC 15min-TWA exposures

Process	**RWC 15min-TWA inhalation exposure (mg/m^3)**	**MOS based on the human 1h-NOAEC for eye irritation of 935 mg/m^3**	**Conclusion**

Manufacture of monomer	65	14.4	(ii)
Production of polystyrene	65	14.4	(ii)
Production of UP-styrene resin	216.5	4.3	(ii)
Production of SBR and SB latex	65	14.4	(ii)
GRP manufacture	779	**1.2**	**(iii)**

For the majority of the scenarios (manufacture of monomer, production of polystyrene and production of SBR and SB latex), the MOS values obtained for the 8h-TWA and short-term exposures are $\geq$ 20 and $\geq$ 14.4 respectively. These MOSs are considered to provide sufficient reassurance that either eye irritation that might be caused by a working day exposure to styrene vapour or eye irritation that might be caused by peak exposures to the vapour will not occur, even allowing for human individual variability in the sensitivity for this effect. Conclusion (ii) is therefore reached for these scenarios.

For production of UP-styrene resin the MOSs obtained for the 8h-TWA and short-term exposures are 5 and 4.3 respectively. Although these MOSs are relatively low, and at least for the 8h-TWA exposure value, based on a borderline LOAEC rather than a clear NOAEC, they are still considered to provide sufficient reassurance that eye irritation that might be caused by a working day exposure to styrene vapour or by peak exposures to the vapour will not occur as there are no obvious kinetic elements for this type of effect to be taken account of. Furthermore, since the starting point for risk characterisation of this endpoint were NOAEC values identified from human data, some interindividual variability in dynamics should have already been accounted for by the heterogeneity of the study groups. Conclusion (ii) is therefore proposed for this scenario in relation to both the 8h-TWA and short-term exposures.

For GRP manufacture the MOSs obtained for the 8h-TWA and short-term exposures are 1 and 1.2 respectively. These MOSs are considered to be too low to account for interindividual variability in the sensitivity for this effect indicating that a working day exposure or peak exposures to styrene vapour in this scenario may cause eye irritation. **Conclusion (iii)** is therefore reached for this scenario only in relation to both the 8h-TWA and short-term exposures.

Respiratory tract irritation

NOAEC values of 100 ppm (433 mg/m^3) for 7 hours and of 216 ppm (935 mg/m^3) for 1-hour have been identified for respiratory tract irritation in humans. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 216 ppm for 1 hour was not a clear NOAEC as one out of nine volunteers reported nasal irritation.

A comparison of these NOAEC values with the RWC 8h-TWA and short-term exposures respectively for the different scenarios is shown in **tables 4.28** and **4.29**.

Table 4.28: MOSs for respiratory tract irritation based upon the RWC 8h-TWA exposures

Process	**RWC 8h-TWA inhalation exposure (mg/m^3)**	**MOS based on the human 7h-NOAEC for respiratory tract irritation of 433 mg/m^3**	**Conclusion**

Manufacture of monomer	4.33	100	(ii)
Production of polystyrene	4.33	100	(ii)
Production of UP-styrene resin	86.6	5	(ii)
Production of SBR and SB latex	21.65	20	(ii)
GRP manufacture	433	**1**	**(iii)**

Table 4.28: MOSs for respiratory tract irritation based upon the RWC 15min-TWA exposures

Process	**RWC 15min-TWA inhalation exposure (mg/m^3)**	**MOS based on the human 1 h-NOAEC for respiratory tract irritation of 935 mg/m^3**	**Conclusion**
Manufacture of monomer	65	14.4	(ii)
Production of polystyrene	65	14.4	(ii)
Production of UP-styrene resin	216.5	4.3	(ii)
Production of SBR and SB latex	65	14.4	(ii)
GRP manufacture	779	**1.2**	**(iii)**

For the majority of the scenarios (manufacture of monomer, production of polystyrene and production of SBR and SB latex), the MOS values obtained for the 8h-TWA and short-term exposures are $\geq$ 20 and $\geq$ 14.4 respectively. These MOSs are considered to provide sufficient reassurance that either respiratory tract irritation that might be caused by a working day exposure to styrene or respiratory tract irritation that might be caused by peak exposures will not occur, even allowing for human individual variability in the sensitivity for this effect. Conclusion (ii) is therefore reached for these scenarios.

For production of UP-styrene resin the MOSs obtained for the 8h-TWA and short-term exposures are 5 and 4.3 respectively. Although these MOSs are relatively low, and at least for the 15min-TWA exposure value, based on a borderline LOAEC rather than a clear NOAEC, they are still considered to provide sufficient reassurance that respiratory tract irritation that might be caused by a working day exposure to styrene or by peak exposures will not occur as there are no obvious kinetic differences for this type of effect to be taken account of. Furthermore, since the starting point for risk characterisation of this endpoint were NOAEC values identified from human data, some interindividual variability in dynamics should have already been accounted for by the heterogeneity of the study groups. Conclusion (ii) is therefore proposed for this scenario in relation to both the 8h-TWA and short-term exposures.

For GRP manufacture the MOSs obtained for the 8h-TWA and short-term exposures are 1 and 1.2 respectively. These MOSs are considered to be too low to account for interindividual variability in the sensitivity for this effect indicating that a working day exposure or peak

exposures to styrene vapour in this scenario may cause respiratory tract irritation. **Conclusion (iii)** is therefore reached for this scenario only in relation to both the 8h-TWA and the short-term exposures.

Repeated dose toxicity

In relation to repeated dose toxicity, risk characterisation should be performed for ototoxicity against the internal NAEL of 300 mg/kg/day identified in rats exposed to styrene for 4 weeks and for effects on colour vision discrimination against a NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA) identified from human studies.

To assess the total body burden for workers exposed in the different scenarios, the RWC dose absorbed via the respiratory tract in an 8h-working day has been combined with the dose of the vapour absorbed via the skin and also with the absorbed dermal dose of the liquid. The body burden deriving from absorption of styrene vapour via the respiratory tract for a 70 kg worker has been estimated assuming that 10 m^3 of air is inhaled in a working day (light activity) and that 100% of the inhaled dose is absorbed. Since dermal absorption of styrene vapour contributes approximately 5% to the total amount absorbed via the respiratory tract and skin combined, the dose absorbed via the respiratory tract represents only 95% of the total amount of the vapour absorbed. This total has therefore been calculated by multiplying the dose absorbed via the respiratory tract by 100/95. The body burden deriving from the RWC dermal exposure to liquid styrene for a 70 kg worker has been estimated by taking into account the predicted exposed surface area for the different scenarios and that only 2% of the dermal dose is absorbed. The total body burden has then been calculated by adding the two estimates together.

The body burdens arising from the RWC inhalation and dermal exposures in each different worker exposure scenario, and the resultant MOSs derived from comparison with the internal NAEL of 300 mg/kg/day for ototoxicity in the rat are shown in **Table 4.29**.

Table 4.29: Body burdens and MOSs for ototoxicity

Scenario	RWC 8h-TWA inhalation exposure (mg/m^3)	RWC 8h inhalation body burden including dose of the vapour absorbed via the skin (mg/kg/day)	RWC 8h dermal exposure to the liquid (mg/day)	RWC 8h dermal body burden (mg/kg/day)	RWC 8h total body burden (mg/kg/day)	MOS based on the rat NAEL for ototoxicity of 300 mg/kg/day	Conclusion
Manufacture of monomer	4.33	0.65	42	0.012	0.662	453	(ii)
Production of polystyrene	4.33	0.65	42	0.012	0.662	453	(ii)
Production of UP-styrene resin	86.6	13	84	0.024	13.024	**23**	**(iii)**
Production of SBR and SB latex	21.65	3.26	42	0.012	3.272	92	(ii)

GRP manufacture	433	65	6560	1.87	66.87	**4.5**	**(iii)**

For the majority of the scenarios (manufacture of monomer, production of polystyrene and production of SBR and SB latex), the MOS values for ototoxicity are ≥ 92. These MOSs are considered to provide sufficient reassurance that ototoxic effects will not occur following chronic exposure to styrene, even after allowing for variability in kinetics and dynamics between and within species, for the irreversibility of the effect and for the fact that the NOAEC was identified in a 4-week study. It is considered that no additional factor for duration adjustment (from subacute to chronic exposure) is necessary in this case as the available evidence has shown that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. Overall, therefore, conclusion (ii) is reached for these scenarios in relation to ototoxicity.

For production of UP-styrene resin the MOS for ototoxicity is 23. It is considered that this value does not provide enough reassurance that ototoxic effects will not occur following chronic exposure to styrene after allowing for variability in kinetics and dynamics between and within species. Although there is specific evidence indicating that the kinetic (at identical exposure concentrations, styrene blood levels in rats and humans are very similar) and dynamic (the anatomical and histological structure of the cochlea in rats and humans is very similar) differences between rats and humans for this effect are likely to be smaller than normally assumed, due to the irreversibility of the effect, it is more prudent to conclude that the MOS obtained for this scenario is not sufficient. **Conclusion (iii)** is therefore proposed. If the typical inhalation (13 mg/m^3) and dermal (8.4 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 1.962 (1.96+0.002) mg/kg/day which is 153 below the rat NAEL of 300 mg/kg/day. This MOS does provide sufficient reassurance that ototoxic effects will not occur in this scenario under typical exposure conditions. Therefore, although conclusion (iii) is proposed for production of UP-styrene resin in relation to RWC exposures, there are no concerns for typical exposures.

For GRP manufacture the MOS is 4.5, which indicates that workers exposed chronically to RWC conditions may be at risk of experiencing ototoxic effects in this scenario and therefore **conclusion (iii)** is reached. If the typical inhalation (173 mg/m^3) and dermal (984 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 26.28 (26+0.28) mg/kg/day which is 11 below the rat NAEL of 300 mg/kg/day. This MOS does not provide sufficient reassurance that ototoxic effects will not occur in this scenario even under typical exposure conditions, and therefore **conclusion (iii)** is also reached for typical exposures.

In relation to effects on colour vision discrimination, the human NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA; equivalent to an internal NAEL of about 32.6 mg/kg/day) identified for these effects has been compared with the body burdens arising from workplace exposures. The body burdens arising from the RWC inhalation and dermal exposures in each different worker exposure scenario, and the resultant MOSs derived from comparison with the human NAEL for effects on colour vision discrimination are shown in **Table 4.30**.

Table 4.30: Body burdens and MOSs for effects on colour vision discrimination

Scenario	RWC 8h-TWA inhalation exposure	RWC 8h inhalation body burden including	RWC 8h dermal exposure to the liquid	RWC 8h dermal body burden	RWC 8h total body burden (mg/kg/day)	MOS based on the human NAEL for effects on colour vision	Conclusion

	(mg/m^3)	dose of the vapour absorbed via the skin (mg/kg/day)	(mg/day)	(mg/kg/day)		discrimination of 32.6 mg/kg/day	
Manufacture of monomer	4.33	0.65	42	0.012	0.662	49.2	(ii)
Production of polystyrene	4.33	0.65	42	0.012	0.662	49.2	(ii)
Production of UP-styrene resin	86.6	13	84	0.024	13.024	2.5	(ii)
Production of SBR and SB latex	21.65	3.26	42	0.012	3.272	10	(ii)
GRP manufacture	433	65	6560	1.87	66.87	**0.5**	**(iii)**

For the majority of the scenarios (manufacture of monomer, production of polystyrene and production of SBR and SB latex), the MOS values for effects on colour vision discrimination are ≥ 10. These MOSs are considered to provide sufficient reassurance that effects on colour vision discrimination will not occur, even after allowing for human individual variability in kinetics and dynamics. Therefore conclusion (ii) is reached for these scenarios in relation to effects on colour vision discrimination.

For production of UP-styrene resin the MOS is 2.5. This value would not normally provide enough reassurance that effects on colour vision discrimination will not occur after allowing for human individual variability. However, given that the starting point for risk characterisation of this endpoint was a NOAEC identified from human studies involving large samples of workers, some interindividual variability should have already been accounted for by the heterogeneity of the study groups. Overall, therefore, it is reasonable to conclude that the MOS obtained for this scenario is sufficient. Conclusion (ii) is proposed in relation to effects on colour vision discrimination.

For GRP manufacture the MOS is 0.5, which indicates that workers exposed to RWC conditions may be at risk of experiencing impairment of colour vision discrimination in this scenario and therefore **conclusion (iii)** is reached. If the typical inhalation (173 mg/m^3) and dermal (984 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 26.28 (26+0.28) mg/kg which is 1.2 below the human NAEL of 32.6 mg/kg. This MOS does not provide sufficient reassurance that effects on colour vision discrimination will not occur in this scenario even under typical exposure conditions, and therefore **conclusion (iii)** is also reached for typical exposures.

Developmental toxicity

In relation to developmental toxicity, a NOAEC of 150 ppm (650 mg/m^3) was identified in a well-conducted rat 2-gen study based upon pup delayed growth (reduced body weights, delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and reductions in forelimb grip strength). Although at 150 ppm there was a decrease in pup

body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. However, this information will be taken into account when judging the adequacy of the Margins of Safety. For comparison with the body burdens arising from workplace exposures, this NOAEC has been converted to an internal NAEL based on the Sarangapani PBPK model oral dose estimate of about 120 mg/kg/day, which generated peak blood levels of styrene that closely matched the predicted blood level of styrene from inhalation exposure to the NOAEC of 150 ppm.

The body burdens arising from the RWC inhalation and dermal exposures in each different worker exposure scenario (calculated as above), and the resultant MOSs derived from comparison with the internal NAEL for pup delayed growth in the rat are shown in **Table 4.31**.

Table 4.31: Body burdens and MOSs for pup delayed growth

Scenario	RWC 8h-TWA inhalation exposure (mg/m^3)	RWC 8h inhalation body burden including dose of the vapour absorbed via the skin (mg/kg/day)	RWC 8h dermal exposure to the liquid (mg/day)	RWC 8h dermal body burden (mg/kg/day)	RWC 8h total body burden (mg/kg/day)	MOS based on the rat NAEL for pup delayed growth of 120 mg/kg/day	Conclusion
Manufacture of monomer	4.33	0.65	42	0.012	0.662	181	(ii)
Production of polystyrene	4.33	0.65	42	0.012	0.662	181	(ii)
Production of UP-styrene resin	86.6	13	84	0.024	13.024	**9**	**(iii)**
Production of SBR and SB latex	21.65	3.26	42	0.012	3.272	**37**	**(iii)**
GRP manufacture	433	65	6560	1.87	66.87	**1.8**	**(iii)**

For manufacture of the monomer and production of polystyrene, the MOS values for pup delayed growth are $\geq$ 181. It is considered that these MOSs provide sufficient reassurance that developmental toxicity will not occur following exposure of pregnant women to styrene in these scenarios, even after allowing for variability in kinetics and dynamics between and within species and for the fact the 150 ppm was not a clear NOAEC. Overall, therefore, conclusion (ii) is reached for these scenarios.

For production of SBR and SB latex, the MOS value for pup delayed growth is 37. This value does not provide sufficient reassurance that developmental toxicity will not occur following exposure of pregnant women to styrene in this scenario, after allowing for variability in kinetics and dynamics between and within species and for the fact the 150 ppm was not a

clear NOAEC. **Conclusion (iii)** is therefore drawn. If the typical inhalation (4.33 mg/m^3) and dermal (21 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 0.626 (0.62+0.006) mg/kg/day which is a factor of 192 below the rat NAEL of 120 mg/kg/day. This MOS does provide sufficient reassurance that developmental toxicity will not occur in this scenario under typical exposure conditions. Therefore, although conclusion (iii) is proposed for production of SBR and SB latex in relation to RWC exposures, there are no concerns for typical exposures.

For production of UP-styrene resin the MOS for pup delayed growth is 9. This value does not provide sufficient reassurance that developmental toxicity will not occur following exposure of pregnant women to styrene in this scenario, after allowing for variability in kinetics and dynamics between and within species and for the fact the 150 ppm was not a clear NOAEC. **Conclusion (iii)** is therefore proposed. If the typical inhalation (13 mg/m^3) and dermal (8.4 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 1.962 (1.96+0.002) mg/kg/day which is 61 below the rat NAEL of 120 mg/kg/day. This MOS does provide sufficient reassurance that developmental toxicity will not occur in this scenario under typical exposure conditions. Therefore, although conclusion (iii) is proposed for production of UP-styrene resin in relation to RWC exposures, there are no concerns for typical exposures.

For GRP manufacture the MOS for pup delayed growth is 1.8, which indicates that developmental toxicity may occur in this scenario following exposure of pregnant women to RWC conditions and therefore **conclusion (iii)** is reached. If the typical inhalation (173 mg/m^3) and dermal (984 mg/day) exposure values are considered for this scenario, the total body burden is estimated at 26.28 (26+0.28) mg/kg/day which is 4.6 below the rat NAEL of 120 mg/kg/day. This MOS does not provide sufficient reassurance that developmental toxicity will not occur in this scenario even under typical exposure conditions, and therefore **conclusion (iii)** is also reached for typical exposures.

Summary of risk characterisation for workers

The MOSs for acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear and colour vision discrimination following repeated exposure and developmental toxicity for the GRP manufacture scenario are unacceptably low, and therefore conclusion (iii) is reached. Conclusion (iii) is also reached for the production of UP-resin scenario in relation to effects on the ear following repeated exposure and developmental toxicity and for production of SBR and SB latex in relation to developmental toxicity.For all remaining scenarios, the MOSs for all of these effects are considered to be sufficient, and therefore conclusion (ii) is proposed.

4.1.3.3 Consumers

Consumer exposures to styrene may be looked at in two ways; a long term but probably very low level exposure, and sporadic and short lived exposures relating to particular events or activities.

4.1.3.3.1 Scenarios involving long-term low level exposures

Long-term low level exposures arise as a result of emissions from polymeric building materials, including carpets, from food sources (mainly as a consequence of food packaging) and from chewing gum. **Table 4.32** gives calculations of body burdens from

these sources. Body burdens have been calculated using the following assumptions: inhalation and oral absorption is 100%; an adult consumer weighs 70 kg.

Table 4.32 Estimated body burdens for consumers, for long-term low level exposures to styrene

Source of exposure	Exposures/Daily intakes of styrene (µg/day)	Estimated body burden (µg/kg/day)
Emissions from polymeric building materials including carpets (inhaled)	5 µg/m^3 = 80 µg/day	1.1
Food (swallowed)	3 µg/day	0.04
Chewing gum (swallowed)	8 µg/day	0.1

Comparison between exposure and effects

Acute toxicity and irritation

The nature of these exposures is such that there are no concerns for acute toxicity or for local irritation. Conclusion (ii) is reached.

Repeated dose toxicity

In relation to repeated dose toxicity, risk characterisation should be performed for ototoxicity against a NOAEC of 500 ppm (2165 mg/m^3) for 4 weeks identified in the rat and for effects on colour vision discrimination against a NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA) identified from human studies. In addition, risk characterisation should be performed for hepatotoxicity against the oral NOAEL of 150 mg/kg/day identified from a 2-year cancer bioassay in the mouse. But in extrapolation of this oral NOAEL to humans careful consideration has to be taken of the specifics of mouse metabolism and the high sensitivity of this species for liver toxicity as compared to e.g. the rat.

In relation to ototoxicity, a NOAEC of 500 ppm (2165 mg/m^3) for 4 weeks exposure (equivalent to an internal NAEL of about 300 mg/kg/day based on the Sarangapani PBPK modelling) has been identified in the rat. For an adult, the maximum total body burden from any individual source is 1.1 µg/kg/day from emissions from polymeric building materials including carpets. This exposure level is at least 5 orders of magnitude lower than the rat NAEL for effects on the ear (MOS=273,000), and does not give rise to concern. All other individual sources of exposure result in lower body burdens and thus higher MOSs. Overall, conclusion (ii) is reached for all of the above consumer exposure scenarios for ototoxic effects.

In relation to effects on colour vision discrimination, the human NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA) has been converted to an internal NAEL of about 32.6 mg/kg/day. For an adult, the maximum total body burden from any individual source is 1.1 µg/kg/day from emissions from polymeric building materials including carpets. This exposure level is at least 4 orders of magnitude lower than the human NAEL for effects on colour vision discrimination (MOS=30,000), and does not give rise to concern. All other individual sources of exposure result in lower body burdens and thus higher MOSs. Overall, conclusion (ii) is reached for all of the above consumer exposure scenarios for effects on colour vision discrimination.

In relation to hepatotoxicity, a NOAEL of 150 mg/kg/day has been identified in an oral cancer bioassay in the mouse. For an adult, the maximum total body burden from any individual source is 1.1 µg/kg/day from emissions from polymeric building materials including carpets. This exposure level is at least 5 orders of magnitude lower than the oral mouse NOAEL for hepatotoxicity (MOS=136,363) and does not give rise to concern. All other individual sources of exposure result in lower body burdens and thus higher MOSs. Overall, conclusion (ii) is reached for all of the above consumer exposure scenarios in relation to hepatotoxicity.

Developmental toxicity

In relation to developmental toxicity, a NOAEC of 150 ppm (650 mg/m^3) was identified in a well-conducted rat 2-gen study based upon pup delayed growth (reduced body weights, delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and small reductions in forelimb grip strength). Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. However, this information will be taken into account when judging the adequacy of the Margins of Safety. For comparison with the body burdens arising from consumer exposures, the NOAEC has been converted to an internal NAEL based on the Sarangapani PBPK model oral dose estimate of about 120 mg/kg/day, which generated peak blood levels of styrene that closely matched the predicted blood level of styrene from inhalation exposure to the NOAEC of 150 ppm. For an adult, the maximum total body burden from any individual source is 1.1 µg/kg/day, from emissions from polymeric building materials including carpets. This exposure level is at least 5 orders of magnitude lower than the rat NAEL for developmental effects (MOS=109,000), and does not give rise to concern. All other individual sources of exposure result in lower body burdens and thus higher MOSs. Overall, conclusion (ii) is reached for all of the above consumer exposure scenarios for potential developmental toxicity.

Summary of risk characterisation for consumers for long-term low level exposure scenarios

Table 4.33 summarises the results of the risk characterisation for consumers for long-term low level exposure scenarios

Table 4.33 Summary of risk characterisation conclusions for consumer scenarios involving long-term low level exposures

Source of exposure	Endpoint				
	Acute toxicity and irritation	**Repeated dose toxicity - ototoxicity**	**Repeated dose toxicity – effects on colour vision discrimination**	**Repeated dose toxicity - hepatotoxicity**	**Developmental toxicity**
Emissions from polymeric	(ii)	(ii)	(ii)	(ii)	(ii)

materials including carpets (inhaled)					
Food (swallowed)	(ii)	(ii)	(ii)	(ii)	(ii)
Chewing gum (swallowed)	(ii)	(ii)	(ii)	(ii)	(ii)

4.1.3.3.2 Combined consumer exposure for long-term low level exposures

The estimated combined exposure (i.e. exposed via emissions from polymeric building materials, via food and from chewing gum) is about 90 µg/day.

Comparison between exposure and effects

Acute toxicity and irritation

The nature of these exposures is such that there are no concerns for acute toxicity or for local irritation. Conclusion (ii) is reached.

Repeated dose toxicity

In relation to repeated dose toxicity, risk characterisation should be performed for ototoxicity against a NOAEC of 500 ppm (2165 mg/m^3) for 4 weeks identified in the rat, for effects on colour vision discrimination against a NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA) identified from human studies and for hepatotoxicity against the oral NOAEL of 150 mg/kg/day identified in a chronic study in the mouse.

In relation to ototoxicity, a NOAEC of 500 ppm (2165 mg/m^3) for 4 weeks exposure (equivalent to an internal NAEL of about 300 mg/kg/day based on the Sarangapani PBPK modelling) has been identified in the rat. For an adult, the combined body burden is about 90 µg/day (equivalent to about 1.2 µg/kg/day). This exposure level is at least 5 orders of magnitude lower than the rat NAEL for effects on the ear (MOS=250,000), and does not give rise to concern. Overall, conclusion (ii) is reached for combined consumer exposure in relation to ototoxic effects.

In relation to effects on colour vision discrimination, the human NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA) has been converted to an internal NAEL of about 32.6 mg/kg/day. For an adult, the combined body burden is about 90 µg/day (equivalent to about 1.2 µg/kg/day). This exposure level is at least 4 orders of magnitude lower than the human NAEL for effects on colour vision discrimination (MOS=27,000), and does not give rise to concern. Overall, conclusion (ii) is reached for combined consumer exposure in relation to effects on colour vision discrimination.

In relation to heapatotoxicity, an oral NOAEL of 150 mg/kg/day has been identified in a chronic study in the mouse. For an adult, the combined body burden is about 90 µg/day (equivalent to about 1.2 µg/kg/day). This exposure level is at least 5 orders of magnitude lower than the mouse NOAEL for hepatotoxicity (MOS=125,000), and does not give rise to

concern. Overall, conclusion (ii) is reached for combined consumer exposure in relation to hepatotoxicity.

Developmental toxicity

In relation to developmental toxicity, a NOAEC of 150 ppm (650 mg/m^3) has been identified in a well-conducted rat 2-gen study based upon pup delayed growth (reduced body weights, delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and small reductions in forelimb grip strength). Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. However, this information will be taken into account when judging the adequacy of the Margins of Safety. For comparison with the body burdens arising from consumer exposures, the NOAEC has been converted to an internal NAEL based on the Sarangapani PBPK model oral dose estimate of about 120 mg/kg/day, which generated peak blood levels of styrene that closely matched the predicted blood level of styrene from inhalation exposure to the NOAEC of 150 ppm. For an adult, the combined body burden is about 90 µg/day (equivalent to about 1.2 µg/kg/day). This exposure level is at least 5 orders of magnitude lower than the rat NAEL for pup delayed growth (MOS=100,000), and does not give rise to concern. Overall, conclusion (ii) is reached for combined consumer exposure in relation to potential developmental toxicity.

Summary of risk characterisation for combined consumer exposure

Conclusion (ii) has been reached for combined consumer exposure in relation to all the endpoints of concern (acute CNS depression, eye and respiratory tract irritation, effects on the ear, colour vision discrimination and hepatotoxicity following repeated exposure and developmental toxicity).

4.1.3.3.3 Scenarios involving short-term infrequent exposures

Short-term exposures can arise as a result of emissions from newly laid carpets, from the use of styrene containing resins for filling or repair of wood, glass fibre or metal, from the use of styrene-based paste and from boat building. **Table 4.34** gives calculations of body burdens from these sources. Body burdens have been calculated using the following assumptions: inhalation absorption is 100%; dermal absorption is 2%; an adult consumer weighs 70 kg.

Table 4.34 Estimated body burdens for consumers, for short-term infrequent exposure to styrene

Source of exposure	Intake of styrene per event (mg)	Estimated body burden (mg/kg/event)	Estimated total body burden (mg/kg/event)
New carpet (inhaled)	2	0.03	0.03
Liquid resin (inhaled)	413	5.9	9.0
Liquid resin (on the skin surface)	11,000	3.1	

Resin paste (inhaled)	68	1	2.6
Resin paste (on the skin surface)	5,500	1.6	
Boat building (inhaled)	4,330	62	62.5
Boat building (on the skin surface)	1,640	0.5	

Comparison between exposure and effects

Acute toxicity

In relation to CNS depression, no effects were seen in humans exposed to 100 ppm (433 mg/m^3) for 7 hours, equivalent to an internal NAEL of about 57 mg/kg.

The total body burdens arising from the inhalation and dermal exposures in each different consumer exposure scenario, and the resultant MOSs derived from comparison with the internal human NAEL for acute CNS depression are shown in **Table 4.35**.

Table 4.35: Body burdens and MOSs for acute CNS depression

Scenario	Total body burden (mg/kg/event)	MOS based on human NAEL for CNS depression of 57 mg/kg	Conclusion
New carpets	0.03	1900	(ii)
Use of liquid resin	9.0	6	(ii)
Use of resin paste	2.6	22	(ii)
Boat building	62.5	0.9	**(iii)**

The MOS for laying of new carpets is 1900. This MOS is very large and hence conclusion (ii) is reached for this scenario in relation to CNS depression.

The MOS values for use of liquid resin and resin paste are 6 and 22, respectively. These MOSs are considered to provide sufficient reassurance that CNS depressant effects will not occur, after allowing for human individual variability in kinetics and dynamics. Therefore conclusion (ii) is reached for these scenarios.

The MOS for the boat building scenario is 0.9. This MOS does not provide sufficient reassurance that CNS depressant effects will not occur, after allowing for human individual variability in kinetics and dynamics. Therefore **conclusion (iii)** is reached for this scenario.

Irritation

Skin irritation

The estimates of exposure to the skin in laying of new carpets indicate that dermal exposures are low. Thus there are no concerns for skin irritation in these scenarios, and conclusion (ii) is reached.

The estimates of exposure to the skin in consumer uses involving handling liquid resin and resin paste suggest that significant skin exposure could occur, and thus there could be concerns for skin irritation. Although it is recognised that there are considerable uncertainties in these exposure estimates, from a practical point of view, the present C&L (R38) should be sufficient to conclude that skin irritation is unlikely to occur in these scenarios. Thus, conclusion (ii) is proposed.

The estimates of exposure to the skin in the boat building scenario indicate that dermal exposures are relatively high. However, from a practical point of view, the present C&L (R38) should be sufficient in preventing skin irritation occurring in this scenario. Thus conclusion (ii) is proposed.

Eye irritation

For exposure to styrene vapour, NOAEC values of 100 ppm (433 mg/m^3) for 7 hours and of 216 ppm (935 mg/m^3) for 1-hour have been identified from exposure of human volunteers. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 100 ppm for 7 hours was not a clear NOAEC for eye irritation as sensations of mild and transient eye dryness were reported at this exposure concentration. A comparison of these NOAEC values with the relevant inhalation exposure estimates for the different scenarios is shown in **table 4.36**.

Table 4.36: MOSs for eye irritation from exposure to the vapour

Scenario	Inhalation exposure concentrations (mg/m^3)	MOS based on human 7h-NOAEC for eye irritation of 433 mg/m^3	MOS based on human 1h-NOAEC for eye irritation of 935 mg/m^3	Conclusion
New carpets (24 hours event)	0.0084	51,548	n.a.	(ii)
Use of liquid resin	318	1.4	3	**(iii)**
Use of resin paste	52	8	18	(ii)
Boat building (8 hours event)	433	1	2.15	**(iii)**

n.r.: not reliable; n.a.: not applicable

For laying of new carpets, the MOS for eye irritation is very large (51,548); thus conclusion (ii) is reached.

For the scenario involving handling resin paste, the MOSs for eye irritation are 8 and 18. These MOSs are considered to be sufficient to account for interindividual variability in the sensitivity for this effect. Therefore, conclusion (ii) is proposed.

For the scenario involving handling liquid resin, the MOSs for eye irritation are 1.4 and 3. These MOSs are too low to account for interindividual variability in the sensitivity for this effect. Therefore, **conclusion (iii)** is proposed.

For boat building, the MOSs for eye irritation are 1 and 2.15. These MOSs are too low to account for interindividual variability in the sensitivity for this effect.Therefore, **conclusion (iii)** is proposed.

Respiratory tract irritation

For exposure to styrene vapour, NOAEC values of 100 ppm (433 mg/m^3) for 7 hours and of 216 ppm (935 mg/m^3) for 1-hour have been identified for respiratory tract irritation in humans. However, when evaluating the adequacy of the resultant Margins of Safety, it should be borne in mind that 216 ppm for 1 hour was not a clear NOAEC as one out of nine volunteers reported nasal irritation.

A comparison of these NOAEC values with the relevant inhalation exposure estimates for the different scenarios is shown in **table 4.37**.

Table 4.37: MOSs for respiratory tract irritation from exposure to the vapour

Scenario	Inhalation exposure concentrations (mg/m^3)	MOS based on human 7h-NOAEC for respiratory tract irritation of 433 mg/m^3	MOS based on human 1h-NOAEC for respiratory tract irritation of 935 mg/m^3	Conclusion
New carpets (24 hours event)	0.0084	51,548	n.a.	(ii)
Use of liquid resin	318	1.4	3	**(iii)**
Use of resin paste	52	8	18	(ii)
Boat building (8 hours event)	433	1	2.15	**(iii)**

n.a.: not applicable

For laying of new carpets, the MOS for respiratory tract irritation is very large (51,548); thus conclusion (ii) is reached.

For the scenario involving handling resin paste, the MOSs for respiratory tract irritation are 8 and 18. These MOSs are considered to be sufficient to account for interindividual variability in the sensitivity for this effect. Therefore, conclusion (ii) is proposed.

For the scenario involving handling liquid resin, the MOSs for respiratory tract irritation are 1.4 and 3. These MOSs are too low to account for interindividual variability in the sensitivity for this effect. Therefore, **conclusion (iii)** is proposed.

For boat building, the MOS for respiratory tract irritation are 1 and 2.15. These MOSs are too low to account for interindividual variability in the sensitivity for this effect.Therefore, **conclusion (ili)** is proposed.

Repeated dose toxicity

In relation to repeated dose toxicity, risk characterisation should be performed for ototoxicity against a NOAEC of 500 ppm (2165 mg/m^3; equivalent to an internal NAEL of about 300 mg/kg/day based on the Sarangapani PBPK modelling) for 4 weeks identified in the rat and

for effects on colour vision discrimination against a NOAEC of 50 ppm (216.5 mg/m^3; 8h-TWA; equivalent to an internal NAEL of about 32.6 mg/kg/day) identified from human studies.

The total body burdens arising from the inhalation and dermal exposures in each different consumer exposure scenario, and the resultant MOSs derived from comparison with the internal rat NAEL for ototoxicity are shown in **Table 4.38**.

Table 4.38: Body burdens and MOSs for ototoxicity in consumer scenarios involving short-term, infrequent exposures

Scenario	Total body burden (mg/kg/event)	MOS based on rat NAEL for ototoxicity of 300 mg/kg	Conclusion
New carpets	0.03	10,000	(ii)
Use of liquid resin	9.0	33	**(iii)**
Use of resin paste	2.6	115	(ii)
Boat building	62.5	4.8	**(iii)**

The MOS for laying of new carpets is 10,000. This MOS is very large and hence conclusion (ii) is reached for this scenario in relation to ototoxicity.

The MOS for use of liquid resins is 33. It is considered that this value does not provide enough reassurance that ototoxic effects will not occur following repeated exposure to styrene after allowing for variability in kinetics and dynamics between and within species and for the irreversibility of the effect. Thus, **conclusion (iii)** is reached for use of liquid resins in relation to ototoxicity.

The MOS for use of resin paste is 115. This MOS is considered to provide sufficient reassurance that ototoxic effects will not occur following repeated exposure to styrene, even after allowing for variability in kinetics and dynamics between and within species and for the irreversibility of the effect. Thus, conclusion (ii) is reached for use of resin paste in relation to ototoxicity.

The MOS for the boat building scenario is 4.8. This MOS is very low and hence, **conclusion (iii)** is reached for this scenario in relation to ototoxicity.

The total body burdens arising from the inhalation and dermal exposures in each different consumer exposure scenario, and the resultant MOSs derived from comparison with the internal human NAEL for effects on colour vision are shown in **Table 4.39**.

Table 4.39: Body burdens and MOSs for effects on colour vision in consumer scenarios involving short-term, infrequent exposures

Scenario	Total body burden (mg/kg/event)	MOS based on human NAEL for effects on colour vision of 32.6 mg/kg/day	Conclusion
New carpets	0.03	1,087	(ii)

Use of liquid resin	9.0	3.6	**(iii)**
Use of resin paste	2.6	12.5	(ii)
Boat building	62.5	0.52	**(iii)**

The MOS for laying of new carpets is 1,087. This MOS is very large and hence conclusion (ii) is reached for this scenario in relation to effects on colour vision.

The MOS for use of liquid resins is 3.6. It is considered that this value does not provide enough reassurance that effects on colour vision will not occur following repeated exposure to styrene after allowing for variability in kinetics and dynamics within species. Thus, **conclusion (iii)** is reached for use of liquid resins in relation to effects on colour vision.

The MOS for use of resin paste is 12.5. This MOS is considered to provide sufficient reassurance that effects on colour vision will not occur following repeated exposure to styrene, even after allowing for variability in kinetics and dynamics within species. Thus, conclusion (ii) is reached for use of resin paste in relation to effects on colour vision.

The MOS for the boat building scenario is 0.52. This MOS is very low and hence, **conclusion (iii)** is reached for this scenario in relation to effects on colour vision.

Developmental toxicity

Since effects on development can be induced during rather short time windows, risk characterisation should also be conducted for this endpoint.

A NOAEC of 150 ppm (650 mg/m^3) has been identified in a well-conducted rat 2-gen study based upon pup delayed growth (reduced body weights, delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and small reductions in forelimb grip strength). Although at 150 ppm there was a decrease in pup body weight, since this was small (up to 10%), limited to the pre-weaning period of the F_2 generation only and not accompanied by other related effects, it was not considered sufficient to set the NOAEC for effects on pups at the next lower exposure concentration. However, this information will be taken into account when judging the adequacy of the Margins of Safety. For comparison with the body burdens arising from consumer exposures, this NOAEC has been converted to an internal NAEL based on the Sarangapani PBPK model oral dose estimate of about 120 mg/kg/day, which generated peak blood levels of styrene that closely matched the predicted blood level of styrene from inhalation exposure to the NOAEC of 150 ppm.

The total body burdens arising from the inhalation and dermal exposures in each different consumer exposure scenario, and the resultant MOSs derived from comparison with the internal rat NAEL for developmental toxicity are shown in **Table 4.40**.

Table 4.40: Body burdens and MOSs for developmental toxicity in consumer scenarios involving short-term, infrequent exposures

Scenario	Total body burden (mg/kg/event)	MOS based on rat NAEL for delayed pup development of 120 mg/kg	Conclusion

New carpets	0.03	4,000	(ii)
Use of liquid resin	9.0	13	**(iii)**
Use of resin paste	2.6	46	**(iii)**
Boat building	62.5	1.9	**(iii)**

The MOS for laying of new carpets is 4,000. This MOS is very large and hence conclusion (ii) is reached for this scenario.

The MOS values for the remaining scenarios are ≥ 1.9. These values are considered not to provide enough reassurance that developmental effects will not occur after allowing for variability in kinetics and dynamics between and within species and for the fact that the 150 ppm is not a clear NOAEC. Thus, **conclusion (iii)** is reached for these scenarios.

Summary of risk characterisation for consumers for short-term infrequent exposure scenarios

Table 4.41 summarises the results of the risk characterisation for consumers for short-term infrequent exposure scenarios.

Table 4.41 Summary of risk characterisation conclusions for consumer scenarios involving short-term infrequent exposures

Source of exposure	Acute toxicity	Skin irritation from exposure to the liquid	Eye and respiratory tract irritation from exposure to the vapour	Repeated dose toxicity – ototoxicity and effects on colour vision	Developmental toxicity
New carpets	(ii)	(ii)	(ii)	(ii)	(ii)
Use of liquid resin	(ii)	(ii)	**(iii)**	**(iii)**	**(iii)**
Use of resin paste	(ii)	(ii)	(ii)	(ii)	**(iii)**
Boat building	**(iii)**	(ii)	**(iii)**	**(iii)**	**(iii)**

4.1.3.4 Indirect exposure via the environment

The key health effects of potential concern in relation to indirect exposures via the environment are repeated dose toxicity (effects on hearing and on colour vision discrimination and hepatotoxicity) and developmental toxicity. Acute toxicity and irritation are of low concern where exposure is dissipated throughout the environment.

4.1.3.4.1 Regional exposure

Based on modelled data, daily human intake via the environment at the regional level is $1.6x10^{-5}$ mg/kg/day (approx. 30-40% from oral sources and 60-70% from air). This level is several orders of magnitude below the rat internal NAEL of 300 mg/kg/day for ototoxicity, the human internal NAEL of 32.6 mg/kg/day for effects on colour vision discrimination, the mouse NOAEL of 150 mg/kg/day for hepatotoxicity and the rat internal NAEL of 120 mg/kg/day for developmental toxicity. Therefore there are no concerns for these endpoints and conclusion (ii) is reached.

4.1.3.4.2 Local exposure

The highest local environmental exposure, predicted from modelled data, occurs in the locality of a styrene production plant and is estimated to be 0.11 mg/kg/day (approx 30-40% from oral sources and 60-70% from air). Based on measured data, the estimated body burden is 0.058 mg/kg/day. It should be noted that this estimate is dominated by the contribution from air. The modelled estimate is 2727 below the rat internal NAEL of 300 mg/kg/day for ototoxicity, 296 below the human internal NAEL for effects on colour vision discrimination, 1363 below the mouse NOAEL for hepatotoxicity and 1090 below the rat internal NAEL for developmental toxicity. Even higher MOS values would result if the measured exposure data were used. These MOSs are considered to be sufficient to provide reassurance that adverse health effects would not occur even after allowing for potential differences in toxicokinetics and toxicodynamics within and between species. Conclusion (ii) is therefore reached.

4.1.3.5 Combined exposure

For combined exposure, consideration should be given to a consumer, who is also exposed via the environment. The most appropriate consumer exposures to consider are those giving rise to long-term, repeated exposures ie. exposures from the release of residual styrene monomer from polymeric styrene products, from food and from chewing gum. These combined consumer exposures result in a daily intake of about 90 µg/day, or 1.3 µg/kg/day for a 70 kg adult. Based on modelled data, the highest exposure via the environment would be to someone living in the vicinity of a styrene production plant, with an estimated daily intake of 0.11 mg/kg/day. The resultant combined exposure would be dominated by the environmental exposure of 0.11 mg/kg/day. If measured environmental exposure data are considered, the daily intake via environmental sources is estimated to be 0.058 mg/kg/day; this would give a combined consumer and environmental intake estimate of 0.059 mg/kg/day.

The key health effects of potential concern in relation to combined exposure are repeated dose toxicity (effects on hearing and on colour vision discrimination and hepatotoxicity) and developmental toxicity. Acute toxicity and irritation are of low concern where exposure is dissipated throughout the environment. The modelled estimate of 0.11 mg/kg/day is 2727 below the rat internal NAEL of 300 mg/kg/day for ototoxicity, 296 below the human internal NAEL for effects on colour vision discrimination, 1363 below the mouse NOAEL for hepatotoxicity and 1090 below the rat internal NAEL for developmental toxicity. Even higher MOS values would result if the measured exposure data (estimate of 0.059 mg/kg/day) were used. These MOSs are considered to be sufficient to provide reassurance that adverse health effects would not occur even after allowing for potential differences in toxicokinetics and toxicodynamics within and between species. Conclusion (ii) is therefore reached.

4.2 HUMAN HEALTH (PHYSICOCHEMICAL PROPERTIES) (RISK ASSESSMENT CONCERNING THE PROPERTIES LISTED IN ANNEX IIA OF REGULATION 1488/94)

4.2.1 Risk characterisation

The flash point of styrene measured by the closed cup method is 31°C. This value is within the range of flammability. Therefore, styrene is classified with R10 (flammable).

5 RESULTS

5.1 GENERAL

5.2 ENVIRONMENTAL RISKS

5.3 RISKS TO HUMAN HEALTH

The hazardous properties of styrene have been evaluated to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of acute toxicity (CNS depression), skin, eye and respiratory tract irritation, repeated dose toxicity and developmental toxicity have been identified. There are no concerns for sensitisation, mutagenicity, carcinogenicity or effects on fertility.

5.4 WORKERS

Five occupational scenarios have been identified: manufacture of the monomer, production of UP-resins, production of polystyrene, production of SBR and SB latex and GRP manufacture.

Result

(X) **(iii)** There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

The MOSs for acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear and colour vision discrimination following repeated exposure and developmental toxicity for GRP manufacture are unacceptably low, and therefore conclusion (iii) applies. Conclusion (iii) also applies to production of UP-resin in relation to effects on the ear following repeated exposure and developmental toxicity and to production of SBR and SB latex in relation to developmental toxicity.

(X) **(ii)** There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

For all remaining scenarios (manufacture of the monomer and production of polystyrene), the MOSs for acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear and colour vision discrimination following repeated exposure and developmental toxicity are considered to be sufficient, and therefore conclusion (ii) applies. Comclusion (ii) also applies to all scenarios in relation to sensitisation, mutagenicity, carcinogenicity and effects on fertility.

5.5 CONSUMERS

Consumer exposure to styrene can arise as a result of emissions from polymeric building materials, including carpets, from food sources (mainly as a consequence of food packaging), from chewing gum, from newly laid carpets, from the use of styrene containing resins for filling or repair of wood, glass fibre or metal, from the use of styrene-based paste and from boat building.

Result

(X) **(iii)** There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to exposures arising from boat building in relation to acute CNS depression, eye and respiratory tract irritation, effects on the ear and on colour vision following repeated exposure and developmental toxicity. Conclusion (iii) also applies to the use of styrene containing liquid resins in relation to eye and respiratory tract irritation, effects on the ear and on colour vision following repeated exposure and developmental toxicity, and to the use of styrene-based paste in relation to developmental toxicity.

(X) **(ii)** There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to long-term low level exposures arising as a result of emissions from polymeric building materials, including carpets, from food sources (mainly as a consequence of food packaging) and from chewing gum in relation to acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear, colour vision discrimination and hepatotoxicity following repeated exposure and developmental toxicity. Conclusion (ii) also applies to exposures arising as a result of emissions from newly laid carpets in relation to acute CNS depression, skin, eye and respiratory tract irritation, effects on the ear following repeated exposure and developmental toxicity. Conclusion (ii) applies to the use of styrene containing liquid resins in relation to acute CNS depression, skin, to the use of styrene-based paste in relation to acute CNS depression, skin, eye and respiratory tract irritation and effects on the ear following repeated exposure and to exposures arising from boat building in relation to skin irritation. Conclusion (ii) applies for all scenarios in relation to sensitisation, mutagenicity, carcinogenicity and effects on fertility.

5.6 INDIRECT EXPOSURE VIA THE ENVIRONMENT

Result

(X) **(ii)** There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to both regional and local exposures in relation to all endpoints.

5.7 COMBINED EXPOSURE

For combined exposure, consideration should be given to a consumer, who is also exposed via the environment. The most appropriate consumer exposures to consider are those giving rise to long-term, repeated exposures ie. exposures from the release of residual styrene monomer from polymeric styrene products, from food and from chewing gum.

Result

(X) **(ii)** There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to combined exposure in relation to all endpoints.

5.8 RISKS FROM PHYSICOCHEMICAL PROPERTIES

There are no significant risks from physicochemical properties.

Result

(X) **(ii)** There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

6 REFERENCES

Agrawal, Srivastava SP, Seth PK (1982)
Effect of styrene on dopamine receptors.
Bull Environ Contam Toxicol. **29;** 400-403.

Ahlborg, G, Bjerkedal, T and Egenaes, J (1987)
Delivery outcome among women employed in the plastics industry in Sweden and Norway.
Am J Indust Med. **12;** 507-517.

Alarie, Y (1973)
Sensory irritation of the upper airways by airborne chemicals.
Toxicol Appl Pharmacol. **24**; 279

Alarie, Y (1981)
Dose-response analysis in animal studies: prediction of human responses.
Environ Health Persp. **42;** 9.

Albee, RR, et al. (1992)
Ototoxicologic and neurotoxicologic evaluation of rats exposed to styrene for 13 weeks.
The Dow Chemical Company Report HET K-000874-036B.

Alieva, ZA, Sultanov, MIu, Mirzoev, TA (1985)
[Impairment of color perception acuity caused by styrene and tetrachloroethylene vapors.] Gig Tr Prof Zabol, **Feb (2)**, 11-3 [Article in Russian].

American Society of Testing Methods, (1939)
Part 3, Nonmetallic materials-General, p.87-88.

Andersson, HC Tranberg, EA, Uggla, AH and Zetterberg, G *et al* (1980)
Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory.
Mutat Res. **73;** 387-401.

Anttila, A, Pukkala, E, Sallmen, M and Hemminki, K (1998)
Cancer incidence among Finnish workers exposed to aromatic hydrocarbons.
Int Arch Occup Environ Health. **71;** 187-193.

Anwar, WA and Shamy, MY (1995)
Chromosomal aberrations and micronuclei in reinforced plastics workers exposed to styrene
Mutat Res. **327**; 41-47.

Artuso, M, Angotzi, G, Bonassi, S, *et al* (1995)
Cytogenetic biomonitoring of styrene-exposed plastic boat builders
Arch Environ Contamin Toxicol. **29;** 270-274.

ARW (1985)
Arbeitsgemeinschaft Rhein-Wasserwerke eV. 42 Bericht.

ARW (1986)
Arbeitsgemeinschaft Rhein-Wasserwerke eV. 43 Bericht.

ARW (1987)
Arbeitsgemeinschaft Rhein-Wasserwerke eV. 44 Bericht.

ARW (1988)
Arbeitsgemeinschaft Rhein-Wasserwerke eV. 45 Bericht.

Ashford R D (1994)
Dictionary of Industrial Chemicals. Wavelength Publications, London.

Asmundo CN, Cataldi Lup MC, Lanza CM, Russo C (1986)
Impiego dei serbatoi in PRFV per la vinificazione in alcune zone della Sicilia orientale.
Vignevini. **12;** 43-33.

Atkinson R, Aschmann SM, Fitz DR, Winer AM and Pitts Jr JN (1982)
Rate constants for the gas phase reactions of O3 with selected organics at 296 K.
Int J Chem Kinet. **14;** 13-18.

Atkinson R (1985)
Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions.
Chem Rev. 69-201.

Axelson, O and Gustavson, J (1978)
Some hygenic and clinical observations on styrene exposure. *Scand J Work Environ Health*. **4**(suppl.2); 215-219.

Bachman, S, Hellwig, J, Jackh, R et al (1998)
Uterotrophic assay on two concentrations of migrates from each of 23 polystyrenes administered orally (by gavage) to immature female Wistar rats.
Drug Chem Toxicol. **21;** 1-30.

Bakke, OM and Scheline, RR (1970)
Hydroxylation of aromatic hydrocarbons in the rat.
*Toxicol. Appl. Pharmaco*l, **16**, 691-700.

Bakhtizina, GZ and popuchiev, VV (1981)
Effect of styrene on compensatory reductive processes in the ovaries of mature rats.
Sb Nauch Tr Mosk NII Gigieny. **11;** 117-122.

Bakhtizina, GZ and Khakimov, BV (1982)
Methodical approaches to the analysis of morphological changes in the neuroendocrine system under the prolonged influence of styrene.
Gigiena Truda I Okhrana Zdorov'ya Rabochikh v Neftegazodobyv.

Bakhtizina, GZ, Koval'skii, GB and Popuchiev, VV (1983)
Morphofunctional study of the ovaries in a long-term styrol exposure.
Gig Sanit. **3;** 77-79.

Banerjee S, Yallowsky SH, Valvani SC (1980)
Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility-partition coefficient correlation.
Environ Sci Technol. **14;** 1227-1229.

Barale, R (1991)
The genetic toxicology of styrene and styrene oxide.
Mutat Res. **257;** 107-126.

Bardodej Z and Bardodejova E (1970)

Biotransformation of ethyl benzene, styrene and alpha-methylstyrene in man.
Am Ind Hyg Assoc J. **31;** 206-9.

Bartels MJ, Rick DL, Zhang F and Kan L (unpublished, 2004).
In vitro metabolism of 4-vinylphenol and styrene in mouse, rat and/or human microsomes obtained from lung and/or liver tissue.
DOW Study No. 031097. The Dow Chemical Company, Midland, MI 48674, USA.

Bartolucci, GB *et al* (1986)
Biomonitoring of occupational exposure to styrene.
Appl Ind hyg. **1;** 125-131.

BASF AG (1987)
Verteilundskoeffizient POW und Hentry-Konstante von Styrol, Bericht BRU 87.206, dated 05.10.1987/du.

BASF AG (1988)
Ecological lab, unpublished data (Ber.v.07.07.88).

BASF AG (1989)
Analytical laboratory report, unpublished data (1/1989).

BASF AG (1990)
Material Safety Data Sheet for styrene, dated 4.90.

BASF AG (2001a)
Unpublished preliminary report: Styrene – subacute vapour inhalation study in female CD1 mice investigations on oxidative stress and cytotoxicity in the lung.
BASF Arkiengesellschaft, FRG.

BASF AG (2001b)
Unpublished preliminary information on the cytogenetic study (micronucleus test) in vivo in mice after inhalation of styrene vapour.
BASF Aktiengesellschaft, FRG.

Basrotti M, Parmeggiani L and Sassi C (1952)
Observations on occupational pathology in a polystyrene resin factory.
Med Lav. **43;** 418-24.

Bauer, C, Leporini, C, Bronzetti, G et al (1980)
Results for styrene in the *in vitro* mutagenesis test with metabolic activation (microsomal assay).
Boll soc It Biol Sper. **56;** 203-207.

Bauer, C, Leporini, C, Bronzetti, G *et al* (1980)
The problem of negative results for styrene in the *in vitro* mutagenesis test with metabolic activation (microsomal assay)
Boll Soc It Biol Sper. **56;** 2200-2205.

Beije, B and Jensen, D (1982)
Investigation of styrene in the liver perfusion/cell culture system, no indication of styrene-7,8-oxide as the principal mutagenic metabolite produced by the intact rat liver.
Chem Biol Interactions. **39;** 57-76.

Beliles, RP, Butala, JH, Stack, CR and Makris, S (1985)

Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats.
Fund Appl Toxicol. **5;** 855-868.

Belverdere, G and Tursi, F (1981)
Styrene oxidation to styrene oxide in human blood erythrocytes and lymphocytes.
Res Comm Chem Path Pharamcol. **33;** 273-282.

Belverdere, G, Elovaara, E and Vainio, H (1984)
Activation of styrene to styrene oxide in hepatocytes and subcellular fractions of rat liver
Toxicol Lett. **23;** 157-162.

Benignus VA, Geller AM, Boyes WK and Bushnell PJ (2005)
Human neurobehavioural effects of long-term exposure to styrene: a meta-analysis.
EHP; **113(5)**: 532-538.

Bergamaschi, E, Smargiassi, A, Mutti, A *et al* (1995)
Immunological changes among workers occupationally exposed to styrene.
Int Arch Occ Environ Health. **67;** 165-171.

Bergamaschi, E, Mutti, A Cavazzini, S *et al* (1996)
Peripheral markers of neurochemical effects among styrene-exposed workers
Neurotoxicol **17;** 753-760.

Bergamaschi, E, Smargiassi, A, Mutti, A et al (1997)
Peripheral markers of catecholamine dysfunction and symptoms of neurotoxicity among styrene-exposed workers.
Int Arch Occup Environ Health. **69;** 209-214.

Bergman K (1979)
Whole body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents.
Scand J work Environ Health. **Suppl 1**; 93-120.

Bernauer U, Heinrich-Hirsch B, Tonnies M, Wolski P and Gundert-Remy U (2005)
Cytochrome P450 2E1 (CYP2E1)-dependent chlorzoxazone hydroxylase activities in human lung microsomes.
Naunyn-Schmiedebergs Arch Pharmacol. 371 (Suppl. 1); R93.

Berode, M, Droz, PO and Guilleman, M (1985)
Human exposure to styrene. IV Percutaneous absorption in human volunteers.
Int Arch Occup Environ Health. **55;** 331-336.

Bigbee, WL, Grant, SG, Langlois, RG et al (1996)
Glycophorin A somatic cell mutation frequencies in Finnish reinforced plastics workers exposed to styrene.
Canc Epidemiol Biomark Prevent. **5;** 801-810.

Bignozzi CA, Maldotti A, Chiorboli C, Bartocci C and Carassiti V (1981)
Kinetics and mechanism of reactions between aromatic olefins and hydroxyl radicals.
Int J Chem Kinetics. **13;** 1235-1242.

Bingquing, C *et al* (1989)
Neurobehavioural effects of styrene inhalation exposure on rat offspring.
Chinese J Preventative Med. **23;** 342-344.

Biró, A, Pallinger, E, Major, J, et al. (2002)
Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed solvents.
Immunology Letters. **81**, 133-140.

Bodell, WJ, Pongracz, K, Kaur, S et al (1990)
Investigation of styrene oxide-DNA adducts and their detection in workers exposed to styrene
Mutation and the Environment, Part C, pp 271-282.

Boffetta, P, Sali, D, Kolstad, H et al (1998)
Mortality of short-term workers in two international cohorts.
JOEM. **40;** 1120-1126.

Bol J, verhaar HJM, Van Leeuwen CJ, Hermens JLM (1993)
Predictions of the aquatic toxicity of high-production-volume chemicals.
Publikatiereeks Stoffen, Veiligheid, Straling, Netherlands, nr 1993/9A.

Boland PA (1981)
Report to Office of Drinking Water, US EPA. Contract No 68-01-192.

Bond, JA (1989)
Review of the toxicology of styrene
CRC Crit rev Toxicol. **19;** 227-249.

Bond, GG *et al* (1992)
Mortality among workers engaged in the development or manufacture of styrene-based products – an update.
Scand J Environ Health. **18;** 145-154.

Bondarevskaya, EP (1957)
Effect of styrene on female genitals in the conditions of production and experiment.
Trudy Voronehjkovo Med Instit. **29;** 11-13.

Boogaard, PJ et al., (2000)
Quantification of DNA adducts formed in liver, lungs and isolated lung cells of rats and mice exposed to C-14-styrene by nose-only inhalation.
Toxicol.Sci. **57**: 203-216

Booth, ED (1999)
Molecular toxicology of styrene: Identification of species differences in the metabolism of styrene in rats and mice.
Unpublished report. Shell Research and Technology centre, Amsterdam. Report number: Not assigned.

Bos R, Guicherit R and Hoogeveen A (1977)
Distribution of some hydrocarbons in ambient air near Delft and the influence of the formation of secondary air pollutants.
Sci Tot Environ **7;** 269-281.

Bos, PMJ, Zwart, A, Reuzel, PGJ and Bragt, PC (1992)
Evaluation of the sensory irritation test for the assessment of occupational health risk.
CRC Crit Rev Toxicol. **21;** 423-450.

Bosner-Cucancic, B et al (1994)
Effects of occupational styrene exposure on haematological parameters.
Toxicol Lett. **Suppl 74/1**; 9.

Boundy and Boyer (1952)
Styrene – its polymers, copolymers and derivatives.
The Dow chemical Co., ACS monograph series, Reinhold Publishing Corporation, NY, USA.

Bouscaren R, frank R and Veldt, C (1986)
Hydrocarbons: Identification of air quality problems in member states of the European Communities.
CEC Environment and Quality of Life. Report EUR 10646 EN.

Bouwer EJ and McCarthy PL (1984)
Modelling of trace organics biotransformation in the subsurface.
Groundwater. **22;** 433-440.

Bovine, LB and Milner, FJM (1963)
Polyester resin hazards.
Br J Ind Med. **20;** 100-9.

Bowman, KJ, Collins, MJ, Henry, CJ (1984)
The effect of age on performance on the panel D-15 and desaturated D-15: a quantitative evaluation.
In: *Color Vision Deficiencies VII* Ed by Verriest G; Dr W Junk Publishers, The Hague, Netherlands pp 227-231.

Brams, A, Buchet, JP, Crutzen-Fayt, MC *et al* (1987)
A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotset.
Toxicol Lett. **38;** 123-133.

Brenner, DB, Jeffrey, AM, Latriano, L et al (1991)
Biomarkers in styrene-exposed boatbuilders.
Mutat Res. **261;** 225-236.

Bridges, BA et al (1994)
Spontaneous mutation and its place in risk assessment for chemical mutagens. Report of an ICPEMC Committee.
Mutat Res. **304;** 3-11.

Bridié AL, Wolff CJM, Winter M (1979a)
BOD and COD of some petrochemicals.
Wat Res. **13;** 627-630.

Bridié AL, Wolff CJM, Winter M (1979b)
The acute toxicity of some petrochemicals to goldfish
Wat Res. **13;** 6230626.

Brighton CA, Pritchard G, Skinner GA (1979)
Styrene polymers: technology and environmental aspects. Applied Science Publishers Ltd, London.
Bringmann, G (1973)

Bestimmung der biologischen Scahdwirkung wassergefährdender Stoffen aus der Hemmung der Glucose-Assimilation des Bakterium Pseudomonas fluorescens.
Gesundheits-Ingenieur. **94(12);** 366-368.

Bringmann, G (1978)
Bestimmung der biologischen Schadwirkung wassergefährdender Stoffen gegen Protzoen I. Bakterienfressende Flagellaten (Modellorganismus: Entosiphon sulcatum Stein).

Bringmann G and Kűhn R (1977a)
Befunde der Schadwirkung wassergefährdender Stoffen gegen Daphnia magna.
Zeitschrift fűr Wasser und Abwasser Forschung. **10;** 161-6.

Bringmann G and Kűhn R (1977b)
Grenzwerte der Schedwirkung wassergefährdender Stoffen gegen Bakterien (Pseudomonas putida) und Grűnalgen (Scenedesums Quadricauda) im Zellvermerhrungshemmtest.
Zeitschrift fűr Wasser und Abwasser Forschung. 87-98.

Bringmann G and Kűhn R (1978)
Grenzwerte der Schedwirkung wassergefährdender Stoffen gegen Blaualgen (Microcystis aeruginosa) und Grűnalgen (Scenedesmus quadricauda) im zellvermehrungshemmtest.
Vom Wasser. **50;** 45-60.

Bringmann G and Kűhn R (1980)
Bestimmung der biologischen Schadwirkung wassergefährdender Stoffen gegen Protzoen III. Bakterienfressende Ciliaten.
Zeitschrift fűr Wasser und Abwasser Forschung. **13;** 26-31

Bringmann G, Kűhn R and Winter A (1980)
Bestimmung der biologischen Scadwirkung wassergefährdender Stoffen gegen Protzoen II. Saprozoische Flagellaten.
Zeitschrift fűr Wasser und Abwasser Forschung. **13;** 170-173.Bringmann G and Kűhn R (1982)
Ergebnisse der Schedworkung wassergefährdender Stoffen gegen Daphnia magna in einem weiterentwickelten standardisierten Testverfahren.
Zeitschrift fűr Wasser und Abwasser Forschung. **15;** 1-6.

Brodzinsky R and Sing HB (1983)
Volatile organic compounds in the atmosphere: An assessment of available data.
EPA/DF – 83/005A. SRI International, Menlo Park, California.

Brooks, SM *et al* (1980)
The effects of protective equipment on styrene exposure in workers in the reinforced plastics industry.
Arch Environ Health. **35;** 287-91

Brown, WR (1999)
SYI/7: Styrene: 104 week repeat dose inhalation combined toxicity/carcinogenicity study in mice. Immunohistochemistry of primary lung tumours.
Unpublished Study Report. Research Pathology Services Inc., PA. USA for The Styrene Information and Research Centre VA, USA.

Brown, NA, Lamb, JC, Brown, SN and Neal, BH (1999)
A review of the developmental and reproductive toxicity of styrene.
Unpublished report of MRC Experimental Embryology and Teratology Unit, University of London.

Brugnone, F, Perbellini, L, Want, GZ (1993)
Blood styrene concentrations in a “normal” population and in exposed workers 16 hours after the end of the workshift.
Int Arch Occup Environ Health. **65;** 125-130.

Brunnemann, K, Rivenson, A, Cheng, S, Saa, V, Hoffmann, D (1992).
A study of tobacco carcinogenesis XLVII. Bioassays of vinylpyridines for genotoxicity and for tumorigenicity in A/J mice.
Cancer Letters, **65**, 107-113.

BUA (1990)
Styrene. Report 48 of the GDCh – Advisory Committee on Existing Substances of Environmental Relevance. VCH, Weinheim, English language version.

Buchanan, SK (1989)
Locating and estimating air emissions from sources of 1,3-butadiene. Final report, Radian corporation, Research Triangle Park, NC. EPA 450/2-89/021.Buckpitt, A et al., (1995)
Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. IV. Metabolism of naphthalene to naphthalene oxide in microdissected airways from mice, rats and hamsters.
Mol. Pharmacol. **47**: 74-81.

Bufalini JJ and Altshuller AP (1965)
Kinetics of vapour-phase hydrocarbon-ozone reactions.
Can J Chem. **43;** 2243-2250.

Burback BL, Perry JP and Rudd LE (1994)
Effect of environmental pollutants and their metabolites on a soil mycobacterium.
Appl Microbiol Biotechnol. 41; 134-136.

Buschini, A, De Palma, G, Poli P, Martino, A, Rossi, C, Mozzoni, P, Scotti, E, Buzio, L, Bergamaschi, E, Mutti, A (2003).
Genetic polymorphism of drug-metabolising enzymes and styrene-induced DNA damage.
Environmental and Molecular Mutagenesis, **41**, 243-252.

Bushnell PJ (1994)
Styrene impairs serial spatial reversal learning in rats.
J Am coll Toxicol. **13;** 279-300.

Busk, L (1979)
Mutagenic effects of styrene and styrene oxide.
Mutat Res **67;** 201-208.Calabrese, G, Martini A, Sessa G *et al* (1996)
Otoneurological study in workers exposed to styrene in the fibreglass industry.
Int Arch Occup Environ Health. **68;** 219-223.

Camurri, L, Codeluppi, S, Pedroni, C and Scarduelli, L (1983)
Chromosomal aberrations and sister-chromatid exchanges in workers exposed to styrene.
Mutat Res. **119;** 361-369.

Camurri, L Codeluppi, S, Scarduelii, L and Candela, S (1984)
Sister-chromatid exchanges in workers exposed to low doses of styrene.
In: Sister Chromatid Exchanges. R.R Trice and A Hollaender (Eds), Plenum NY, pp957-963.

Campagna, D, Mergler, D, Huel, G, Belanger, S, Truchon, G, Ostiguy, C, Drolet, D (1992)
Visual dysfunction among styrene-exposed workers.

Proc Int Symposium Epidemiology Occup Health meeting abstract; Sept 23-25 (Cincinnati, Ohio).

Campagna D, Mergler, D, Huel, G et al (1995)
Visual dysfunction among styrene-exposed workers.
Scand J Work Env Health. **21;** 382-390.

Campagna, D, Gobba, F, Mergler, D et al (1996)
Color vision loss among styrene-exposed workers. Neurotoxicological Threshold Assessment.
NeuroToxicol. **17;** 367-374.

Campo P, Loquet G, Blachere V and Roure M (1999)
Toluene and styrene intoxication route in the rat cochlea.
Neurotoxicol Teratol. **21:** 427-434.

Campo P, Lataye, R, Loquet G and Bonnet P (2001)
Styrene-induced hearing loss: a membrane insult.
Hearing Research **154**: 170-180.

Cantor, KP, Stewart, PA, Brinton, LA and Dosemeci, M (1995)
Occupational exposures and female breast cancer mortality in the United States.
JOEM. **37;** 336-348.

Cantoreggi, S and Lutz, WK (1993)
Covalent binding of styrene to DNA in rat and mouse.
Carcinogenesis. **14:** 355-360.

Caperos, JR, Humbert, B and Droz, PO (1979)
Styrene exposure. II Percentage studies of absorption, excretion and metabolism by human subjects.
Int Arch Occup Environ Health. **42;** 223-30.

Carlson, GP (1997a)
Comparison of mouse strains for susceptibility to styrene-induced hepatotoxicity and pneumotoxicity.
J Toxicol Environ Health. **51;** 177-187.

Carlson, GP (1997b)
Effects of inducers and inhibitors on the microsomal metabolism of styrene to styrene oxide in mice.
J Toxicol Environ Health. **51;** 477-487.

Carlson, GP, Hynes, DE and Mantick, NA (1998)
Effects of inhibitors of CYP1A and CYP2B on Styrene metabolism in mouse liver and lung microsomes.
Toxicol Lett. **98;** 131-137.

Carlson, GP (1999a)
Studies on the metabolism of styrene by isolated lung cells.
Unpublished review for the Styrene Information and Research Center.

Carlson, GP (1999b)
Metabolsim of styrene oxide to styrene glycol by mouse liver and lung.
J. Toxicol. Environ. Health, Part A, **53**, 19-27.

Carlson, GP (2000)
Metabolsim of styrene oxide to styrene glycol in enriched mouse Clara-cell preparations.
J. Toxicol. Environ. Health, Part A, **61**, 709-717.

Carlson, GP, Mantick, NA and Powley, MW (2000)
Metabolism of styrene by human liver and lung.
J Toxicol Environ Health, Part A, **59,** 591-595.

Carlson, GP, Perez Rivera, AA, Mantick, NA (2001)
Metabolism of styrene metabolite 4-vinylphenol by rat and mouse liver and lung.
J. Toxicol. Environ. Health, Part A, **63**, 541-551.

Carlson, GP, Ullman, M, Mantick, NA, Synder PW (2002a)
4-vinylphenol-induced pneumotoxicity and hepatotoxicity in mice.
*Toxicol. Pathol. **30(5)**, 565-569.*

Carlson, GP (2002b)
Effect of the inhibition of the metabolism of 4-vinylphenol on its hepatotoxicity and pneumotoxicity in rats and mice.
Toxicology, **179**, 129-136.

Carlson, GP (2003).
In vitro metabolism of styrene to styrene oxide in liver and lung of CYP2E1 knockout mice.
Journal of Toxicology and Environmental Health, **Part A**, **66**, 861-869.

Carlson, GP (2004)
Comparison of the susceptibility of wild-type and CYP2E1 knockout mice to the hepatotoxic and pneumotoxic effects of styrene and styrene oxide.
Toxicol Lett. **150;** 335-339.
Carpenter, CP et al (1944)
Studies on the inhalation of 1,3-butadiene with a comparison of its narcotic effect with benzol, toluol and styrene.
J Ind Hyg. **26;** 69-78.

Castillo, L, Baldwin, M, Sassine, MP, and Mergler, D (2001)
Cumulative exposure to styrene and visual functions.
Am. J. Ind. Med. **39**, 351-360.

Chaiyavech et al (1959)
J Chem Eng Data **4;** 53-56.

Chakrabarti, S et al (1993)
Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects.
Environ Mol Mutagen. **22;** 85-92.

Challenor, J, and Wright, D (2001)
Aggression in boat builders: a search for altered mood states in boat builders exposed to styrene.
Occup. Med. **50(3)**, 185-192.

Checkoway, H, echeverria, D, Moon, J-D et al (1994)
Platelet monoamine oxidase B activity in workers exposed to styrene.
Int Arch Occup Env Health. **66;** 359-362.

Checkoway, H et al (1992)
Peripheral markers of neurochemical function among workers exposed to styrene.
Brit J Indust Med. **49;** 560-565.

Checkoway, H and Williams, TM (1982)
A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant.
AM Indust Hyg Assoc J **43;** 164-169.

Chem-Intell (1991)
Chefacts: UK, 1991 edition. Chem-Intell, Chemical Intelligence Services, Reed Publishing Ltd, Dunstable.

Chernoff, N *et al* (1990)
Effects of chemically induced maternal toxicity on prenatal development in the rat.
Teratol. **42;** 651-658.

Cherry, N and Gautrin, D (1990)
Neurotoxic effects of styrene: further evidence.
Brit J Indust Med. **47;** 29-37.

Cherry, N *et al* (1981)
Acute behavioural effects of styrene exposure: a further analysis.
Brit J Indust Med. **38;** 346-350.

Cherry, N *et al* (1980)
An investigation of the acute behavioural effects of styrene on factory workers.
Brit J Indust Med. **37;** 234-240.Chia, S-E *et al* (1994)
Impairment of colour vision among workers exposed to low concentrations of styrene.
AM J Indust Med. **26;** 481-488.

Chmielewski J et al (1973)
Rating of the exposure to styrene of persons working at the production of polyesteric laminates.
Bull Inst Marit Trop Med Gdansk. **24;** 203-9.

Chmielewski, J and Renke, W (1976)
Clinical and experimental research into the pathogenesis of toxic effects of styrene. II Morphology, coagulation and fibrinolysis systems of the blood in persons exposed to the action of styrene during their work.
Bull Inst Marit trop Med Gdansk. **27;** 63-8.

Clay, P. (2004)
Styrene monomer does not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure.
Mutagenesis, **19(6)**; 489-492.

CITI (1992)
Biodegradation and bioaccumulation data of existing chemicals based on the CSCL (chemical Substance Control Laboratory, Japan. Edited by Chemicals Inspection and Testing Institute. Japan, October 1992.

Coccini T, Randine G, Li B, Manzo L and costa L G (1999)
Effect of styrene on monoamine oxidase B activity in rat brain.
J Toxicol Environ Health. **56;** 59-68.

Coccini T, Di Nucci A, Tonini M, maestri L, Costa L G, Liuzzi M and Manzo L (1996)
Effects of ethanol administration on cerebral non-protein sulfhydryl content in rats exposed to styrene vapour.
Toxicol. **106;** 115-122.

Coggon, D *et al* (1987)
Mortality of workers exposed to styrene in the manufacture of glass-reinforced plastics.
Scand J Work Environ Health. **13;** 94-99.

Cole, J and Skopek, TR (1994)
Somatic mutant frequency mutation rate and mutational spectra in the human population in vivo
Mutat Res **304;** 33-105.

CONCAWE (1987)
A survey of exposures to gasoline vapour. Report No 4/87. The Hague. The Netherlands.

Conde-Salazar, L *et al* (1989)
Occupational allergic contact dermatitis from styrene.
Contact Dermatitis. **21;** 112.

Conner, MK, Alarie, Y and Dombroske, RL (1979)
Sister chromatid exchange in regenerating liver and bone marrow cells of mice exposed to styrene
Toxicol Appl Pharmacol **50;** 365-367.

Connor, MK, Alarie, Y and Dombroske, RL (1980a)
Sister chromatid exchange in murine alveolar macrophages, bone marrow and regenerating liver cells induced by styrene inhalation.
Toxicol Appl Pharmacol. **55;** 37-42.

Conner, MK, alarie, Y and Dombroske, RL (1980b)
Multiple tissue comparisons of sister chromatid exchanges induced by inhaled styrene.
In: *Genotoxic effects of airborne agents*. Eds Tice RR, costa DL and Schaich. Plenum NY.

Conti, B et al (1988)
Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats and p-methylstyrene administered by ingestion in Sprague Dawley rats and Swiss mice.
Ann NY Acad Sci. **534;** 203-234.

Corsini, E et al (1994)
Styrene inhalation and immune function in mice.
Inhal Toxicol. **6;** 647-654.

Coyle YM, Hynan LS, Euhus DM and Minhajuddin ATM (2005)
An ecological study of the association of environmental chemicals on breast cancer incidence in Texas.
Breast Cancer Research and Treatment; **92**: 107-114.

Crandall MS and Hartle RW (1985)
An analysis of exposure to styrene in the reinforced plastic boat making industry.
AM J Indust Med. **8;** 183-192

Crawford, C (1983)

Styrene taint in hot cross buns: a possible pathway.
FMBR Bulletin No 1; 45-54.

CRC (1994)
Handbook of Chemistry and Physics, 75th edition.

Cruzan, G, Andrews, LS, Cushman, JR *et al* (1993)
Subchronic (13 week) vapour inhalation study of styrene in rats.
Abstract presented at the International Symposium on Health Hazards of Butadiene and Styrene. Espoo, Finland.

Cruzan, G, Cushman, JR, Andrews, LS, Granville, GC, Miller, RR, Hardy, CJ, Coombs, DW and Mullins, PA (1997)
Subchronic inhalation studies of styrene in CD rats and CD-1 mice.
Fund Appl Toxicol. **35;** 152-165.

Cruzan, G Cushman, JR, Andrews, LS *et al* (1998)
Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks.
Toxicol Sci. **46;** 266-281.

Cruzan, G *et al* (2001)
Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks.
J Appl. Toxicol. **21**: 185-198.

Cruzan, G, Carlson, GP, Johnson, KA, Andrews, LS, Banton, MI, Bevan, C and Cushman, JR (2002)
Styrene respiratory tract toxicity and mouse lung tumours are mediated by CYP2F-generated metabolites.
Regulatory Toxicology and Pharmacology, **35**: 308-319.

Cruzan, G, Carlson, G, Turner, M, Mellert W (2005).
Ring-oxidized metabolites of styrene contribute to styrene-induced Clara cell toxicity in mice.
Journal of Toxicology and Environmental Health, Part A. **68**: 229-237.

Cruzan G, Faber WD, Johnson KA, Roberts LS, Hellwig J, Carney E, Yarrington JT and Stump DG (2005a)
Two generation reproduction study of styrene by inhalation in Crl-CD rats.
Birth Defects Research (Part B) **74**: 211-220.

Cruzan G, Faber WD, Johnson KA, Roberts LS, Hellwig J, Maurissen J, Beck MJ, Radovsky A and Stump DG (2005b)
Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats.
Birth Defects Research (Part B) **74**: 221-232.

Csanady GA, Mendrala AL, Nolan RJ and Filser JG (1994)
A physiologic pharmacokinetic model for styrene and styrene-7, 8 oxide in mouse, rat and man.
Arch Toxicol. **68;** 143-157.

Csanady, GA, Kessler, W, Hoffmann, HD and Filser, JG (2003)
A toxicokinetic model for styrene and its metabolite styrene-7,8-oxide in mouse, rat and human with special emphasis on the lung.
*Toxicol Lett. **138**, 75-102*

CSU (1994)
Annual Abstract of Statistics, 1994. Central Statistical Office, HMSO, London.

Dalton, P, Cowart, B, Dilks, D, Gould, M, Lees, P, Stefaniak, A, Emmett, E (2003).
Olfactory function in workers exposed to styrene in the reinforced-plastics industry.
American Journal of Industrial Medicine, **44,** 1-11.

Danishefsky, I, Willhite, M (1954)
The Metabolism of styrene in the rat.
J Biol. Chem. **211;** 549-53.

Darnall KR, Lloyd AC, Winer AM, Pitts JN Jr (1976)
Reactivity scale for atmospheric hydrocarbons based on reaction with hydroxyl radical.
Environ Sci Technol. **10;** 692-696.

Das, M, Dixit, R, Mushtaqu, M *et al* (1981)
Effect of styrene on hepatic mixed function oxidases, glutathione content and glutathione-S-tranferase activity in rats.
Drug Chem Toxicol. **4;** 219-227.

Daston, GP *et al* (1991)
The role of metallothionein induction and altered zinc status in maternally mediated developmental toxicity: comparison of the effects of urethane and styrene in rats.
Toxicol Appl Pharmacol **110;** 450-463.

De Ceaurriz, JC, Micillino, JC, Bonnet, P and Guenier, JP (1981)
Sensory irritation caused by various airborne chemicals.
Toxicol Lett. **9;** 137.

Delbressine, LPC, Van Balderen, PJ, Smeets, FLM and Seutter-Berlage, F (1981)
Stereoselective oxidation of styrene to styrene oxide in rats as measured by mercapturic acid excretion.
Xenobiot. **11;** 589-594.

Del Carratore, R et al (193)
Cytochrome P450 factors determining synthesis in strain D7 of Saccharomyces cerevisiae.
Mutat Res. **121;** 117-123.

De Flora, S (1981)
Study of 106 organic and inorganic compounds in the Salmonella/microsome test.
Carcinogen. **2;** 283-298.

De Flora, S, Zanacchi, P, Camoiranio, A *et al* (1984)
Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test.
Mutat Res. **133;** 161-198.

Delzell, E, Department of Epidemiology, University of Alabama, Birmingham, USA. (Unpublished report, April 2004)
Styrene exposure and mortality from ischaemic heart disease among North American rubber industry workers, 1944-1998.
Report submitted to the Styrene Information Research Center.

Delzell, E, Macaluso, M, Sathiakumar, N, Matthews, R (2001)

Leukaemia and exposure to 1,3-butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry
Chemico-Biological Interactions, **135-136,** 515-534.

Delzell, E, Sathiakuma, N, Hovinga, M *et al* (1996).
A follow-up study of synthetic rubber workers.
Toxicol. **113;** 182-189.

De Meester, C *et al* (1977a)
Mutagenicity of styrene and styrene oxide.
Mutat Res. **56;** 147-52.

De Meester, C, Poncelet, F, Roberfroid, M *et al* (1977b)
Mutagenic activity of styrene and styrene oxide. A preliminary study.
Arch Int Physiol Biochem. **85;** 398-399.

De Meester, C, *et al* (1981)
Mutagenicity of styrene in the *Salmonella typhimurium* test system.
Mutat Res. **90;** 443-450.

De Palma, G, Manini, P, Mozzoni, P, Andreoli, R, Bergamaschi, E, Cavazzini, S, Franchini, I, Mutti, A (2001)
Polymorphism of xenobiotic enzymes and excretion of styrene mercapturic acids.
Chem. Res. Toxicology, **14**, 1393-1400.

De Raat, WK (1978)
Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate.
Chem Biol Interact. **20;** 163-170.

Ding, X. and Kaminsky, L.S. (2003).
Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts.
Annu. Rev. Pharmacol. Toxicol. **43**, 149-173.

Dixit, R, Das, M, Mushtaq, M *et al* (1982)
Depletion of glutathione content and inhibition of glutathione-S-tranferase and aryl hydrocarbon hydroxylase activity in rat brain following exposure to styrene.
NeuroTox. **3;** 142-145.

Dogra, PKS, Khanna, S, Srivatava, SN *et al* (1989)
Styrene-induced immunomodulation in mice.
Int J Immunopharmac. **11;** 577-586.

Dolara, P, Caderni, G, Lodovici, M *et al* (1984)
Determination of styrene in the urine of workers manufacturing polystyrene plastics.
Am Occup Hyg. **28;** 195-199.

Dolmierski, R, Kwiatkowski, SR and Nitka, J (1976)
Clinical and experimental research into the pathogenesis of toxic effects of styrene. VII. Appraisal of the nervous system in the workers exposed to styrene.
Bull Inst Marit Trop Med Gdansk. **27;** 193-196.

Dolmerski, R, Szczepanik, M, Danielewicz-Garbalinska, G *et al* (1983)

Mutagenic action of styrene and its metabolites. I. Chromosome aberration in persons exposed to the actions of styrene.
Bull Inst Marit Trop Med Gyynia. **35;** 89-93.

Donner, M, Sorsa, M and Vainio, H (1979)
Recessive lethals induced by styrene and styrene oxide in Drosophila melanogaster.
Mutat Res. **67;** 373-6

Dreyer et al (1954)
J Prakt Chem. 273.

Dreyer et al (1955)
J Prakt Chem. 324.

Drummond, L, Caldwell, J and Wilson, HK (1989)
The metabolism of ethylbenzene and styrene to mandelic acid: stereochemical considerations.
Xenobiot. **19;** 199-207.

Dumas S, Parent M-E, Siemiatycki J, Brisson J (2000).
Rectal cancer and occupational risk factors: a hypothesis-generating exposure-based case-control study.
International Journal of Cancer, **87**, 874-879.

Dunkel, VC *et al* (1985)
Reproducibility of microbial mutagenicity assays: II Testing of carcinogens and non-carcinogens in Salmonella typhimurium and Escherichia coli.
Environ Mutagen. **7 Suppl. 5;** 1-248.

EACGI (1995)
Personal communication.

ECETOC (1992)
Styrene toxicology investigations on the potential for carcinogenicity Technical report Number 52.

ECETOC (1994)
Styrene draft criteria document.

ECETOC (1994a)
Environmental Exposure Assessment. ECETOC Technical Report 61.

ECN (1995a)
European Chemical News, 3-9 April, p22.

ECN (1995b)
European Chemical News, 17-23 April, p10

ECN (1995c)
European Chemical News, 5-11 June, p16.

ECN (1995d)
European Chemical News, 1-7 May, p11.

ECN (1995e)

European Chemical News, 1-7 May, p27

Edling, C *et al* (1993)
Increase in neuropsychiatric symptoms after occupational exposure to low levels of styrene.
Brit J Ind Med. **50;** 843-850.

Edling, C and Ekberg, K (1985)
No acute behavioural effects of exposure to styrene: a safe level of exposure? *Brit J Ind Med*. **42;** 301-304.

Edwards JR, EK and Edwards Snr, EK (1998)
Unusual cutaneous reaction to polystyrene in an otherwise healthy population.
Contact Dermatitis. **38;** 50.

Eguchi, T, Kishi, R, Harabuchi, I et al (1994)
Colour vision among workers exposed to styrene
JPN J Ind H. **36;** 104-105.

Eguchi, T, Kishi, R, Harabuchi, I *et al* (1995)
Impaired colour discrimination among workers exposed to styrene: relevance of a urinary metabolite.
Occup Environ Med. **52;** 534-538.

Ellemberger, J and Mihn, G (1975) Mutagenic activity of cyclophosphamide, isofoafamide, and trosfosfamide in different genes of Esherichia coli and Salmonella typhimurium after biotransformation through extracts of rodent liver.
Arch Toxicol. **33;** 225-240.

Englehardt, G, Gamer, A, Vodicka, P, Barta, I, Hoffmann, H-D, Veenstra, G (2003).
A re-assessment of styrene-induced clastogenicity in mice in a subacute inhalation study.
Archives of Toxicology, **77**, 56-61.

Engstrom, K et al (1976)
Urinary mandelic acid concentration after occupational exposure to styrene and its use as a biological exposure test.
Scand J Work Environ Health. **2;** 21-26.

Engstrom, J (1978)
Styrene in subcutaneous adipose tissue after experimental and industrial exposure.
Scand J Work Environ Health. **4 suppl. 2;** 119-20.

Engstrom, K, Bjurstrom, R, Astrand, I and Ovrum, P (1978a)
Uptake, distribution and elimination of styrene in man. Concentration in subcutaneous adipose tissue.
Scand J Work Environ Health. **4;** 315-323.

Engstrom, K, Astrand, I and Wegaeus, E (1978b)
Exposure to styrene in a polymerisation plant. Uptake in the organism and concentration in subcutaneous adipose tissue.
Scand J Work Environ Health. **4;** 324-329.

Environment Canada (1993)
Styrene. Priority substances list assessment report. Canadian Environmental Protection Act. Canada Communication Group Publishing. Minister of Supply and Services, Ottawa.

Fail, PA, Hines, JW, Zacharewski, T, *et al* (1998)
Assessment of polystyrene extract for estrogenic activity in the rat uterotrophic model and an *in vitro* recombinant receptor gene assay.
Drug and Chemical Toxicology, **21**, 101-121.

Fallas, C *et al* (1992)
Subclinical impairment of colour vision among workers exposed to styrene.
Brit J Ind Med. **49**; 679-682.

Fallas, C, Fallas, J, Maslard, P, Dally, S (1993)
Subclinical impairment of colour vision among workers exposed to styrene (Reply).
Br J Ind Med **50**, 766-7.

Fechter LD (1993)
Effects of acute styrene and simultaneous noise exposure on auditory function in the guinea pig.*Neurotoxicol Teratol*.**15**; 151-155.
Fernandez, JG, Caperos, JR (1977)
Styrene exposure. 1. An experimental study of pulmonary absorption and excretion in humans. Int. Arch, Occup, Environ. Health. 40, 1-12.

Fielding M, Gibson TM, James HA, McLoughlin K, Steel CP (1981)
Organic micropollutants in drinking water. Water Research Centre, Medmenham. UK
Technical report TR 159.

Filser, JG *et al* (1993)
Species-specific pharmacokinetics of styrene in rat and mouse.
Arch. Toxicol. **67**, 517-530.

Filser, JG, Oberste-Frielinghaus, HR, Dhawan, M *et al* (1999)
A physiological toxicokinetic model for styrene and styrene-7,8-oxide in mouse, rat and human considering metabolism in the lung.
Unpublished, Preliminary Report.

Filser, J (unpublished, 2004).
Styrene-7,8-oxide burden in ventilated, perfused lungs of mice and rats exposed to styrene vapours.
GSF-Institute of Toxicology, D-85764 Neuherberg, Germany.

Fiserova-Bergerova, V and Teisinger, J (1965)
Pulmonary styrene vapour retention.
Ind. Med. Surg. **34**, 620-2.

Fleig, I and Thiess, AM (1978)
Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry.
Scand. J. Work Env. Health. **4(suppl.2)**,254-258.

Flodin, U, Ekbert, K and Andersson, L (1989)
Neuropsychiatric effects of low exposure to styrene.
British J. Ind. Med. **46**, 805-808.

Forkert, PG (1995)
CYP2E1 is preferentially expressed in Clara cells of murine lungs: localisation by in situ hybridization and immunohistochemical methods.
Am. J. Respir. Cell Mol. Biol. **12**: 589-596.

Foster JR (1999a)
Nasal and lung histoautoradiography in male CD rats and male CD mice following a 6-hour inhalation exposure to [^{14}C] styrene.
Unpublished study report. Central Toxicology Laboratory, UK.
Report number: CTL/R/1411

Foster JR (1999b)
The role of cytochrome P450 in styrene induced nasal lesions in the mouse.
Unpublished study report. Central Toxicology Laboratory, UK.
Report number: CTL/R/1418

Foth, H. (1995).
Role of the lung in accumulation and metabolism of xenobiotic compounds – implications for chemically induced toxicity.
Crit. Rev. Toxicol. **25**, 165-205.

Fourman, GL, Harris, C, Guengerich, FP and Bend, JR (1989)
Stereoselectivity of styrene oxidation in microsomes and purified cytochrome-P450 enzymes from rat liver.
The Journal of Pharmacology and Experimental Therapeutics, **248(2)**, 492-496

Frentzel-Beyme, R, Theiss, AM and Wieland, R (1978)
Survey of mortality among employees engaged in the manufacture of styrene and polystyrene at the BASF Ludwigshafen works.
Scand.J. Work. Environ. Health, **4(suppl.2)**, 231-239.

Fu MH and Alexander M (1992)
Biodegradation of styrene in samples of natural environments.
Environ. Sci. Technol., **26**, 1540-1544.

Fumiko W (1988)
Styrene exposure and rat operant behaviour.
Hanshi Ishi **37**, 337-350.

Fung, F and Clark, RF (1999)
Styrene-induced peripheral neuropathy.
Clinical Toxicology **37(1)**, 91-97.

Fustinoni, S, Colosio, C, Colombi, A, et al (1998) Albumin and haemoglobin adducts as biomarkers of exposure to styrene in fibreglass-reinforced-plastics workers.
International archives of Occupational and Environmental Health, **71**, 35-41.

Gadalina, ID *et al* (1969)
Hygienic assessment of polyester plastics for flooring.
Hug. Sanit. **34**, 25-29.

Gadberry, MG, DeNicola, DB and Carlson, GP (1996)
Pneumotoxicity and hepatotoxicity of styrene and styrene oxide.
J Toxicol. Environ, Health, **48**, 273-294

Gamberale, F and Hultegren, M (1974)
Exposure to styrene. II Psychological functions.
Work Environ Health **11**, 86-93.

Gamberale, F, Lisper, HO and Olson, BA (1976)
The effect of styrene vapour on the reaction time of workers in the plastic boat industry.
In adverse effects of environmental chemicals and psychotropic drugs. Horvath M.
(ed.), Amsterdam, Elsevier Scientific Publications. 135-48.

Gamer, AO, Leibold, E, Deckardt, K, Kittel, B, Kaufmann, W, Tennekes, HA and Van Ravenzwaay, B (2004)
The effects of styrene on lung cells in female mice and rats.
Food and Chemical Toxicology, **42**, 1655-1667.

Gedik, L and Roper, CS (2003). The *in vitro* percutaneous absorption of radiolabelled styrene in two test preparations through human skin. Unpublished report. Inveresk report number 20825. Inveresk Research, Tranent EH33 2NE, Scotland.

Geiger DL, Brooke LT and Call DJ (1990)
Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas), Volume 5. Centre for Lake Superior Environmental Studies, University of Wisconsin, United States of America. Pp332.

Geller AM and Hudnell HK (1997)
Critical issues in the use and analysis of the Lanthony Desaturate colour Vision Test.
Neurotoxicology and Teratology **19(6)**, 455-465.

Georgieva, T, Lukanova, A, Panev, T and Popov, T (1998)
Study of erythrocytes, haemoglobin levels, and menstrual cycle characteristics of women exposed to aromatic hydrocarbons.
International Archives of Occupational & Environmental Health, **71(Suppl.)**, S16-18.

Gerin, M, Siemiatycki, J, Desy, M and Krewski, D (1998)
Association between several sites of cancer and occupational exposure to benzene, toluene, xylene and styrene: Results of a case-control study in Montreal.
American Journal of Industrial Medicine, **34**, 144-156.

Geuskens, RBM et al (1992)
Exposure to styrene and health complaints in the Dutch glass reinforced plastics industry.
Ann. Occup. Hyg. **36**, 47-57.

Ghanayem BI, Wang H and Sumner S (2000)
Using cytochrome P-450 gene knock-out mice to study chemical metabolism, toxicity and carcinogenicity.
Toxicol. Pathol. **28**, 839-850.

Gilbert J and Startin JR (1983)
A survey of styrene monomer levels in foods and plastic packaging by coupled mass spectrometry-automatic headspace gas chromatography.
J. Sci. Food Agric., **34**, 647-652.

Girman JR, Hodgson AT, Newton AS, Winnkes AW (1986)
Emissions of volatile organic compounds from adhesives with indoor applications.
Environment International, **12**, 317-321.

Gobba, F *et al* (1991)
Acquired dyschromatopsia among styrene-exposed workers.
Journal of Occupational Medicine. **33**, no. 7, 761-765.

Gobba F, Cavalleri A (1993)
Kinetics of urinary excretion and effects on colour vision after exposure to styrene.
IARC Sci Publ, **127**, 79-88.

Gobba, F *et al* (1993)
Urinary styrene in the biological monitoring of styrene exposure.
Scand. J. work Environ. Health. **19**, 175-182.

Gobba, F, Cavalleri, F, Bontadi, D *et al* (1995)
Peripheral neuropathy in styrene-exposed workers.
Scand J Work Env Health **21**, 517-520.

Gobba, F and Cavalleri, A (2000)
Evolution of color vision loss induced by occupational exposure to chemicals.
Neurotoxicology, **21**, 777-81.

Gong YY, Kishi R, Katakura Y, Tsukishima E, Fujiwara K, Kasai S, Satoh T, Sata F, Kawai T (2002)
Relation between colour vision loss and occupational styrene exposure level.
Occup Environ Med **59**, 824-9.

Grant, WM (1974)
Toxicology of the eye. (2nd ed.) Springfield, Illinois; Charles C. Thomas, 946-948.

Green, T (1999a)
Styrene: A 10 day inhalation study to investigate effects on mouse and rat lung.
Unpublished study report. Central Toxicology Laboratory, UK.
Report number: CTL/R/1346

Green, T (1999b)
The effects of styrene on mouse lung following oral dosing
Unpublished study report. Central Toxicology Laboratory, UK.
Report number: CTL/R/1410

Green, T (1999c)
The metabolism of styrene by rat, mouse and human nasal cytochrome P450s.
Unpublished study report. Central Toxicology Laboratory, UK. Report number: CTL/R/1412.

Green, T (1999d)
The role of cytochrome P450 in styrene induced cell division in the mouse lung.
Unpublished study report. Central Toxicology Laboratory, UK. Report number: CTL/R/1409.

Green, T (2000a)
Styrene metabolism and the distribution of cytochromes P450 2F2 and 2E1 in lung tissue from rat, mouse and human.
Unpublished study report. Central Toxicology Laboratory, UK. Report number: CTL/R/1447.

Green, T (2000b)
The effects of styrene on the mouse lung after exposures of 2, 4 and 6 hours.
Unpublished study report. Central Toxicology Laboratory, UK. Report number: CTL/R/1462.

Green, T (2000c)

The metabolism of styrene oxide in rat, mouse, human nasal epoxide hydrolases and glutathione s-transferases.
Unpublished study report. Central Toxicology Laboratory, UK. Report number: CTL/R/1474.Green T, Toghill A, Foster J (2001a).
The role of cytochromes P-450 in styrene induced pulmonary toxicity and carcinogenicity.
Toxicology, **169**, 107-117.

Green T, Lee R, Toghill A, Meadowcroft S, Lund V, Foster J (2001b).
The toxicity of styrene to the nasal epithelium of mice and rats: studies on the mode of action and relevance to humans.
Chemico-Biological Interactions, **137**, 185-202.

Gregory, J, Foster, K, Tyler, H and Wiseman M (1990).
Dietary and nutritional survey of British adults (mainly tables).
HMSO, London.

Gregory, J, Lowe, S, Bates, CJ, Prentice, A, Jackson, LV, Smithers, G, Wenlock R and Farron, M (2000)
National diet and nutrition survey: young people aged 4 to 18 years, Volume 1: Report of the diet and nutrition survey. The stationery Office, London.

Gregotti, C, Kirby, Z, Manzo, L *et al* (1994)
Effects of styrene oxide on differentiation and viability of rodent embryo cultures.
Toxicol Appl Pharmacol. 128: 25-35.

Greim, H, Bimboes, D, Egert, G *et al* (1977)
Mutagenicity and Chromosomal aberrations as an analytical tool for *in vitro* detection of mammalian enzyme-mediated formation of reactive metabolites.
Arch Toxicol. 39; 159-169.

Grosjean D (1985)
Atmospheric reactions of styrenes and peroxybenzoyl nitrate.
Sci. Tot. Environ., **46**, 41-59.

Grosjean D and Fung K (1984)
Hydrocarbons and carbonyls in Los Angeles air.
J. Air Pollution Control Assoc., **34(5)**, 537-543.

Grosman IG (1970)
Waterborne styrene in a crystalline bedrock aquifer in the Gales Ferry Area, Ledyard, Southeastern Connecticut.
US Geol Surv Prof Pap, 700-B, 203-209.

Guicherit R and Schulting FL (1985)
The occurrence of organic chemicals in the atmosphere of the Netherlands.
Sci. Tot. Environ., **43**, 193-219.

Guillemin, MP and Bauer D (1978)
Biological monitoring of exposure to styrene by analysis of combined urinary mandelic and phenylglyoxylic acids.
Am Ind Hyg Assoc J. **39**, 873-9.

Guilleman, MP and Bauer, D (1979)
Human exposure to styrene. III. Elimination kenetics of urinary mandelic acid and phenylglyoxylic acids after single experimental exposure.

Int. Arch. Occup. Environ. Health. **44**, 249-263.

Hagman, L, Hogstedt, B, Welinder, H et al (1989)
Cytogenetic and hematological effects in plastics workers exposed to styrene.
Scand. J. Work Environ. Health. **15**, 136-141.

Hamilton JD, Reinert KH, Hagan JV and Lord WV (1995)
Polymers as solid waste in municipal landfills.
J. Air and Waste Management Association, **45**, 247-251.

Hansch C and Leo A (1979)
Substituent constant for correlation analysis in chemistry and biology. Pormona College. Wiley-Interscience Publication, John Wiley and Sons, New York.

Hansteen, I-L, Jelmert, O, Torgrimsen, T, et al (1984)
Low human exposure to styrene in relation to chromosome breaks, gaps and sister chromatid exchanges.
Hereditas **100**, 87-91.

Hardy, C J *et al* (1996)
Styrene: 104 week repeat dose inhalation combined toxicity/carcinogenicity study in rats. Volume 1.
Unpublished Study Report. Huntingdon Life Sciences, UK. Report No. SYI 4/943028.

Hardy, CJ *et al* (1993)
Styrene: 13-week repeat dose inhalation study in rats.
Unpublished Study Report. Huntingdon Life Sciences, UK. Report No. SYI 2/920692.

Härkönen, H (1977)
Relationship of symptoms to occupational styrene exposure – and to the findings of electroencephalographic and psychological examinations.
Int. Arch. Occup. Environ. Health. **40**, 231-9.

Härkönen, H, Lindstrom, H and Seppalainen, AM (1978a)
Exposure response relationship between styrene exposure and central nervous function.
Scand. J. work. Environ. Health, **4**, 53-59.

Härkönen, H, Lindstrom, H and Seppalainen, AM (1978b)
Psychological performance and electrocardiographic findings in occupational styrene exposure.
Scand. J. Work. Environ. Health. **4(suppl.2)**, 195-196.

Härkönen, H and Holmberg, PC (1982)
Obstetric histories of women occupationally exposed to styrene.
Scand J Work Environ Health. **8**, 74-77.

Härkönen, H *et al* (1984)
Congenital malformations, mortality and styrene exposure.
Annals Academy of Medicine (Singapore) **13**, 404-407.

Hayes, JP (1991)
Occupational asthma due to styrene.
Thorax **46**, 396-397.

Hazardous Substances Database (1993)

Heaton (1986)
The Chemical Industry. Editor C R Heaton. Blackie and Son, Glasgow.

Heitmüller PT, Hollister TA and Parrish PR (1981)
Acute toxicity of 54 industrial chemicals to sheepshead minnows (Cyprinodon variegatus).
Bull. Environ. Contam. Toxicol., **27**, 596-604.

Hemminki, K, Franssila, E and Vainio, H (1980)
spontaneous abortions among female chemical workers in Finland.
Int. Arch. Occup. Environ. Health. **45**, 123-126.

Hemminki, K (1984)
Reproductive hazards and plastics industry.
In "Industrial Hazards of Plastics and Synthetic Elastomers" 79-87.

Herbert, KL and Sivak, JG (1996)
In Vitro measure of ocular damage and recovery from aromatics.
J. Toxicol.-Cut. & Ocular Toxicol. **15(4)**, 331-341.

Higgins C E, Griest WH and Olerich G (1983)
Application of tenax trapping to analysis of gas phase organic compounds in ultra-low tar cigarette smoke.
J. Assoc. Off. Anal. Chem., **66(5)**, 1074-1083.

Hodgson, JT and Jones, RD (1985)
Mortality of styrene production, polymerisation and processing workers at a site in northwest England.
Scand J. Work Environ. Health **11**, 247-352.

Hogstedt, B, Hedner, K, Mark-Vendel, E *et al* (1979)
Increased frequency of chromosome aberrations in workers exposed to styrene
Scand J. Work Environ. Health **5**, 333-335.

Hogstedt, B, Akesson, B, Axell, K, *et al* (1983)
Increased frequency of lymphocyte micronuclei in workers producing reinforced polyester resin with low exposure to styrene.
Scand J. Work Environ. Health. **9**, 241-246.

Holcomb LC and Seabrook BS (1995)
Indoor concentrations of volatile organic compounds: implications for comfort, health and regulation.
Indoor Environ. **4**, 7-26.

Holmberg, PC (1977)
Central nervous defects in two children of mothers exposed to chemicals in the reinforced plastics industry.
Scand. J. Work. Environ Health. **3**, 212-214.

Holmberg, PC (1978)
Two children with central nervous defects born to mothers exposed to styrene at work.
Scand. J. Work. Environ. Health. **4(suppl. 2)**, 212-214.

Holmberg, PC (1979)
Central nervous system defects in children born to mothers exposed to organic solvents during pregnancy.

Lancet, **2**, 177-179.

Holmberg, PC and Nurminen, M (1980)
Congenital defects to the central nervous system and occupational exposure factors during pregnancy; a case-referent study.
American J. Ind. Med. **1**, 167-176.

Holmberg, PC *et al* (1982)
Oral clefts and organic solvent exposure during pregnancy.
Int. Arch. Occup. Environ. Health. **50**, 371-376.

Holmberg, PC *et al* (1986)
Solvent exposure and birth defects: an epidemiological survey.
Prog. Clin. Biol. Res. **220**, 179-185.

Horvath, E *et al* (1994)
32P-Post-labelling detection of DNA adducts in mononuclear cells of workers occupationally exposed to styrene.
Carcinogenesis. **15**, 1309-1315.

Hoshika Y (1977)
Gas chromatographic determination of styrene as its dibromide.
Journal of Chromatography, **136**, 95-103.

Howard PH, Boethling RS, Jarvis WF, Meylan WM and Michalenko EM (1991) Handbook of Environmental Degradation Rates. Lewis Publishers Inc., Michigan.

Holz, O, Scherer, G, Brodtmeier, S *et al* (1995)
Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant.
Occupational and Environmental Medicine, **52**, 420-428

Hukkanen, J., Pelkonen, O., Hakkola, J. and Raunio, H. (2002).
Expression and regulation of xenobiotic-metabolizing Cytochrome (CYP) enzymes in human lung.
Crit. Rev. Toxicol. **32**, 391-411.

Husain R, Srivastava SP and Seth PK (1985)
Some behavioural effects of early styrene intoxication in experimental animals.
Archives of Toxicology **57**, 53-55.

Husain R, Srivastava SP and Seth PK (1980)
Effects of styrene on levels of serotonin, noradrenaline, dopamine and activity of acetylcholine esterase and monoamine oxidase in rat brain.
Toxicology letters **7**, 47-50.

Hüls AG (1984)
Untersuchungsprotokill Nr 252F. Fischtest 16-4-84.

Hüls AG (1986)
Mod STURM-test No 20. 11/86

Hruba, E, Salemanova, Z and Schwartzova, K (1975)
the long-term investigation of workers at risk from styrene.
Cesk. Neurol. Neurochir. **38**, 116-22.

Huzl, F (1967)
The problem of hazards in working with styrene.
Prac. Lek. **19**, 121-5

Hynes D, DeNicola D, Carlson G (1999).
Metabolism of styrene by mouse and rat isolated lung cells.
Toxicological Sciences, **51**, 195-201.

IISRP (1994)
Worldwide rubber statistics 1994. International Institute of Synthetic Rubber Producers, Inc., Houston, Texas.

Izyumova, AS (1972)
The action of small concentrations of styrol on the sexual functions of albino rats.
Gig. Sanit. **37**, 29-30.

Izyumova, AS, Zlobina, NS and Gabrielyan, NI (1972)
Action of styrene on the reproductive function of rats.
In: Filin, A. P. ed. Vop Gig Tr Profzabol, Mater Nauch Konf 1971: Karaganda USSR Kaz Nauch-Issled Inst Gig Tr Profzabol. 151-152.

Jablonicka, A *et al* (1988)
Analysis of chromosomes in peripheral blood lymphocytes of styrene-exposed workers.
Mutation Research **206**, 167-169.

James, SP, and White, DA (1967)
The metabolism of phenethyl bromide, styrene and styrene oxide in the rabbit and the rat.
Biochem. J. **104**, 914-921.

Jantunen, K, Maki-Paakkanen, J and Norppa, H (1986)
Induction of chromosome aberrations by styrene and vinylacetate in culture human lymphocytes: dependence on erythrocytes.
Mutation Research **159**, 109-116.

Japanese Environment Agency (1991)
Chemicals In The Environment. Report on Environmental Survey and Wildlife Monitoring of Chemicals in FY 1988 and 1989. Office of Health Studies, Department of Environmental Health.

Jarry, H, Metten, M, Gamer, OA and Wuttke W (2002)
Effects of 5-day styrene inhalation on serum prolactine and dopamine levels and on hypothalamic and striatal catecholamine concentrations in male rats.
Arch. Toxicol. **76**, 657-663.

Jarry, H, Gamer, A and Wuttke, W (2004)
Effects of 5-day styrene inhalation on serum LH and testosterone levels and on hypothalamic and striatal amino acid neurotransmitter concentrations in male rats.
Inhalation Toxicology **16**, 209-215.

Jegaden, D, Amann, D, Simon, JF, Habault, M, Legoux, B and Galopin, P (1993)
Study of the neurobehavioural toxicity of styrene at low levels of exposure.
Int. Arch. Occup. Environ. Health. **64**, 527-531.

Jelnes, JE (1988)
Semen quality in workers producing reinforced plastic.

Reproductive Toxicology. **2**, 209-212.

Jersey, GC *et al* (1978)
Two-year chronic inhalation toxicity and carcinogenicity study on monomeric styrene in rats. Report by Toxicology Research Laboratory Dow Chemical Co, USA.

Jickells, SM *et al* (1993)
Migration of styrene monomer from thermoset polyester cookware into foods using high temperature applications.
Food Additives and Contaminants. **10**, 567-573.

Johanson, G, Ernstgard, L, Gullstrand, E, Lof, A, Osterman-Golkar, S, Williams, C, Sumner, S (2000).
Styrene oxide in blood, hemoglobin adducts and urinary metabolites in human volunteers exposed to 13C8-styrene vapors.
Toxicology and Applied Pharmacology, **168**, 36-49.

Johnson AC and Nylen PR (1995)
Effects of industrial solvents on hearing.
Occup Med; **10(3)**.

Jolly AM, Willoughby B, Karas GC and Hobbs SJ (1994)
Use category document: plastics additives. Building Research Establishment, Watford.

Juhnke I and Lüdemann D (1978)
Ergebnise der Untersuchung von 200 chemischen Verbindungen auf akute Fischtoxizität mit dem Goldorfentest.
Zeitschrift fur Wasser und Abwasser Forschung, 11, 161-164.

Jüttner F (1988)
Motor-boat-derived volatile organic compounds (VOC) in lakewater.
Z. Wasser-Abwasser-Forsch., 21, 36-39.

Jüttner F (1994)
Emission of aromatic hydrocarbons and aldehydes into the water by a 4-stroke outboard motor: quantitative measurements.
Chemosphere, **29(2)**, 191-200.

Kankaanpaa, JTJ, Hemminki, K and Vainio, H (1979)
Embryotoxicity and teratogenicity of styrene and styrene oxide on chick embryos enhanced by trichloropropylene oxide.
Acta. Pharamacol. Et Toxicol., **45**, 399-402.

Kankaanpaa, JTJ *et al* (1980)
The effect of maternally inhaled styrene on embryonal and foetal development in mice and Chinese hamsters.
Acta Pharmacologica et Toxicologica. **47**, 127-129.

Karakaya, AE, Karahalil, B, Yilmazer, M *et al* (1997)
Evaluation of genotoxic potential of styrene in furniture workers using unsaturated polyester resin.
Mutation Research, **392**, 261-268.

Katakura, Y, Kishi, R, Ikeda, T and Miyake, H (1999)
Effects of prenatal exposure to styrene on neurochemical levels in rat brain.

Toxicology letters, **105**, 239-249.

Katakura, Y, Kishi, R, Ikeda, T, Miyake, H (2001).
Effects of prenatal styrene exposure on post-natal development and brain and catecholamine levels in rats.
Environmental Research. **A85**, 41-47.

Kats, BY (1962)
Styrene induced toxics-chemical hepatic injury under industrial conditions.
Gig. Tr Prof. Zabol. **6**, 21-24

Kaufmann, W, Mellert, W, van Ravenzwaay, B, Landsiedel, R, Poole, A (unpublished, 2004).
Effects of styrene and its metabolites on different lung compartments of the mouse – cell proliferation and histomorphology.
BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany.

Kelsey, KT *et al* (1990)
Sister-chromatid exchanges in lymphocytes from styrene-exposed boat builders.
Mutation Research. **241**, 215-221.

Kenny, TJ *et al* (1992)
Styrene monomer. 2-week repeat dose inhalation toxicity study in mice
HRC study report SYI 1/920282.

Kenrick MAP, Clark L, Baxter KM, Fleet M, James HA, Gibson TM, Turrell MB (1985)
Trace organics in British aquifers – a baseline survey.
WRC Environment TR 223.

Kessler, W, Jiang, X and Filser, JG (1992)
Pharmacokinetics of styrene-7,8-oxide in mice and rats.
Gentner Verlag. 622-626.

Khanna, VK, Husain, R, Haing, JP and Seth, PK (1991)
Increased Neurobehavioural toxicity of Styrene in protein-malnourished rats.
Neurotoxicology and Teratology, **13**, 153-159.

Khanna VK, Husain R and Seth PK (1994)
Effect of protein malnutrition on the neurobehavioural toxicity of styrene in young rats.
Journal of Applied Toxicology **14**, 251-356.

Kiloh, LG and Osselton, JW (1966)
Clinical electroencephalography. (2^{nd} ed.) London, Butterworth

Kim, H, Wang, RS, Elovaaras, E et al (1997)
Cytochrome P450 isoenzymes responsible for the metabolism of toluene and styrene in human liver microsomes.
Zenobiotica, **27(7)**, 657-665.

Kirk-Othmer (1991)
Encyclopaedia of Chemical Technology, 4^{th} Edition,

Kishi, R *et al* (1989)
Placental transfer and tissue distribution of ^{14}C-styrene: an autoradiographic study in mice.
British J. Ind. Med. **46**, 376-383.

Kishi, R *et al* (1992)
Neurochemical effects in rats following gestational exposure to styrene.
Toxicology Letters. **63**, 141-146.

Kishi, R, Chen, BQ, Katakura, Y *et al* (1995)
Effect of prenatal exposure to styrene on the neurobehavioural development, activity, motor coordination and learning behaviour of rats.
Neurotoxicology and Teratology, **17(2)**, 121-130.

Kishi, R, Eguchi, T, Yuasa, J, Katakura, Y, Arata, Y, Harabuchi, I, Kawai, T, Masuchi, A (2001) Effects of low-level occupational exposure to styrene on color vision: dose relation with a urinary metabolite.
Environ Res, **85**, 25-30.

Kjellberg, A et al (1979)
Long-term effects of exposure to styrene in a polyester plant.
Arbete Och halsa. **18**, 55-67.

Klaerner, P, Klenz, R, Eder, R *et al* (1998)
Preparation and analysis of styrene ologmers containing migrates from various polystyrenes used in food packaging.
Drug and Chemical Toxicology, **12**, 31-49.

Kligerman, AD *et al* (1992)
Cytogenetic studies of mice exposed to styrene by inhalation
Mutation Research. **280**, 35-43.

Kligerman, AD *et al* (1993)
Cytogenetic studies of rodents exposed to styrene by inhalation.
IARC Scientific Publications No. 127, 217-224.

Klimkova-Deutschova, E *et al* (1973)
Recent advances in the neurological picture of occupational exposure to styrene,
Cesk. Neurol, Neurochir. **36**, 20-25.

Kogevinas, M, Ferro, G, Andersen, A *et al* (1994)
Cancer mortality in a historical cohort study of workers exposed to styrene.
Scand. J. Work Environ. Health. **20**, 249-259.

Kolstad, HA *et al* (1994)
Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry.
Scand. J. Work Environ. Health. **20**, 272-278.

Kolstad, HA, Juel, K, Olsen, J and Lynge, E (1995)
Exposure to styrene and chronic health effects: mortality and incidence of solid cancers in the Danish reinforced plastics industry.
Occupational and Environmental Medicine, **52**, 320-327.

Kolstad, HA, Pedersen, B, Olsen, J *et al* (1996)
Clonal chromosomal aberrations in myeloid leukaemia after styrene exposure.
Scand. J. work Environ. Health. **22**, 58-61.

Kolstad, HA, Bisanti, L, Roeleveld, N *et al* (1999a)

Time to pregnancy for men occupationally exposed to styrene in several European reinforced plastics companies.
Scand.J..Work.Environ. Health, **25(suppl. 1)**, 66-69.

Kolstad, HA, Bonde, JPE, Spano, M *et al* (1999b)
Sperm Chromatin structure and semen quality following occupational styrene exposure.
Scand.J.Work.Environ.Health, **25(suppl.1)**, 70-73.

Kolstad, HA, Bonde, JPE, Spano, M *et al* (1999c)
Change in semen quality and sperm chromatin structure following occupational styrene exposure.
International Archives Occupational and Environmental Health, **72**, 135-141.

Kolstad, H.A., Sonderskov, J., Burstyn,I.(2005)
Company-level, semi-quantitative assessment of occupational styrene exposure when individual data are not available.
Annals Of Occupational Hygiene, **49** (2), 155-165

Komaromy L and Tigyi A (1988)
A unique cell type in the lung – the Clara cell (the non-ciliatedbronchiolar pithelial cell).
Acta Biol Hung, **39 (1),** 17-29.

Kondo M, Nishihara T, Shimamoto T, Koshikawa T, I1o T, Sawamura R, Tanaka K (1988)
Biodegradation test of chemicals by cultivation method.
Eisei Kagaku, **34(2)**, 188-195.

Kornm M, Wodraz, R, Drysch, K and Schmah1, FW (1987)
Stereometabolism of styrene in man.
Arch. Toxicol. **68**, 524-527.

Koskinen, MP, Vodicka, P, and Hemminki, K (2001)
Identification of 1-adenine DNA adducts in workers occupationally exposed to styrene.
Journal of Occupational Environmental Medicine, **43**, 694-700.

Krill RM and Sonzogni WC (1986)
Chemical monitoring of Wisconsin's groundwater
J. Amer Water Works Assoc, **78**, 70-75.

Kulig, BM (1989)
The neurobehavioural effects of chronic styrene exposure in the rat.
Neurotoxicology and Teratology. **10**, 511-517.

Kurppa, K *et al* (1983)
Screening for occupational exposures and congenital malformations.
Scand. J. Work Environ. Health. **9**, 89-93.

Laffon, B, Pasaro, E, and Mendez, J (2002)
Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene.
Toxicology, **171**, 175-186.

Lagrone S (1991)
Potential community exposure to toxic chemicals.
Environ. Sci technol., **25(3)**, 366-368.

Landrigan PJ (1993)

Critical assessment of epidemiological studies on the carcinogenicity of 1,3-butadiene and styrene
IARC Scientific Publications No 127 IARC: Lyon, France. 375-388.

Laplanche A, Lemasle M, Laife A and Martin G (1985)
Mesure des émissions de styrène au cours de fabrications de pièces en plastiques armés.
Pollution Atmospherique, **106**, 107-110.

Lambert, B, Bastlova, T, Osterholm, AM and Hou, SM (1995)
Analysis of mutation at the hprt locus in human T lymphocytes.
Toxicology Letters, **82/83**, 323-333.

Lane, W (1946)
Ind. Eng.Chem. (Anal.Ed.), **18**, 295-6.

Lataye R, Campo P and Loquet G (2000)
Combined effects of noise and styrene exposure on hearing function in the rat.
Hearing Research **139**, 86-96.

Lataye R, Campo P, Pouyatos B, Cossec B, Blachere V, Morel G (2003).
Solvent ototoxicity in the rat and guinea pig.
Neurotoxicology and Teratology, **25**, 39-50.

Lataye R, Pouyatos B, Campo P, Lambert A-M and Morel G (2004)
Critical period for styrene ototoxicity in the rat.
Noise & Health, **7(25),** 1-10.

Lataye R, Campo P, Loquet G and Morel G (2005)
Combined effects of noise and styrene on hearing: comparison between active and sedentary rats.
Noise & Health, **7(27)**, 49-64.

Laws RJ, Fileman TW and Matthiessen P (1991)
Phthalate esters and other organic chemicals in the North and Irish Seas.
Water Science and Technology, **24(10)**, 127-134.

Leavens, TL, Farris, GM, H James, RA *et al* (1997)
Genotoxicity and cytotoxicity in male B6C3F_1 mice following exposure to mixtures of 1,3 butadiene and styrene
Environmental and Molecular Mutagenesis, **29**, 335-345.

LeBlanc GA (1980)
Acute toxicity of priority pollutants to water flea (Daphnia magna).
Bull. Environ. Contam. Toxicol., **24**, 684-491.

Lees P, Stefaniak A, Emmett E, Dalton P (2003).
Exposure assessment for study of olfactory function in workers exposed to styrene in the reinforced-plastics industry.
American Journal of Industrial Medicine, **44,** 12-23.

Legraverend, C, Elovaara, E, Zitting, A *et al* (1984)
DNA binding of [14C] styrene in isolated rat hepatocytes.
Chem.-Biol. Interactions, **51**, 91-102.

Leibman, KC and Ortiz, E (1968)

Styrene epoxide – an intermediate in microsomal oxidation of styrene to its glycol.
Pharmacologist, **10**, 203.

Leibman, KC (1975)
Metabolism and toxicity of styrene.
Environ. Health. Perspect. **11**, 115-9.

Lemasters, GK, Hagen, A and Samuels, SJ (1985)
Reproductive outcomes in women exposed to solvents in 36 reinforced plastics companies. I menstrual dysfunction.
Journal of Occupational Medicine. **27(7)**, 490-494.

Lemasters, GK (1989)
Reproductive outcomes of pregnant workers employed at 36 reinforced plastics companies. II Lowered birth weight.
Journal of Occupational Medicine. **31(2)**, 115-120.

Letz, R *et al* (1990)
Neurobehavioural effects of acute styrene exposure in fibreglass boatbuilders.
Neurotoxicology and Teratology. **12**, 665-668.

Lickly, TD *et al* (1995)
A model for estimating the daily dietary intake of a substance from food-contact articles: styrene from polystyrene food-contact polymers.
Regulatory Toxicology and Pharmacology. **21**, 406-417.

Lilis, R *et al* (1978)
Neurotoxicity of styrene in production and polymerisation workers.
Environ Res. **15**, 133-8.

Limasset, JC, Simon, P, Poirot, P *et al* (1999)
Estimation of the percutaneous absorption of styrene in an industrial situation.
International Archives of Occupational and environmental Health, **72**, 46-51.

Lindbohm, M-L, Hemminki, K and Kyyronen, P (1985)
Spontaneous abortions among women employed in the plastics industry.
America Journal Industrial Medicine. **8**, 579-586.

Lindbohm, M-L, Taskinen, H, Sallmen, M et al (1990)
Spontaneous abortions among women exposed to organic solvents.
American Journal of Industrial Medicine. **17**, 449-463.

Lindbohm, M-L (1993)
Effects of styrene on the reproductive health of women. A review. In: Sorsa, M., Peltonen, K., Vainio, H., Hemminki K. (Eds). Butadiene and styrene: Assessment of health hazards, Lyon International Agency for Research on Cancer. Scientific publication Number 127 pp163-169.

Lindbohm, M-L (1999)
Effects of occupational solvent exposure on fertility.
Scand.J.Work environ. Health, **25(suppl. 1)**, 44-46.

Lindstrom, K, Mannila, M, forsman-Gronholm, L *et al* (1992)
Relationships between changes in psychological performances and styrene exposure in work with reinforced plastics.

Tyo ja Ihminen. 6, 2, 181-194. HSE Translation No. 14718D.

Lindstrom, K, Harkonen, H and Hemberg, S. (1976)
Disturbances in psychological function in workers exposed to styrene.
Scand. J. Work. Environ. Health, **3**, 129-139.

Linhart, I, Weidenhoffer, Z, Mraz, J *et al* (1997)
The evidence for conjugated mandelic acid and phenylglyoxylic acids in the urine of rats dosed with styrene.
Toxicology Letters, **90**, 199-205.

Linhart, I, Gut, W, Smejkal, J and Novak, J (2000)
Biotransformation of styrene in mice. Stereochemical aspects.
Chem. Res. Toxicol., **13**, 36-44.

Linnainmaa, K *et al* (1978a)
Cytogenetic effects of styrene and styrene oxide.
Mutation Research. **58**, 277-286.

Linnainmaa, K, Meretoja, T, Sorsa, M and Vainio, H (1978b)
Cytogenetic effects of styrene and styrene oxide on human lymphocytes and Allium cepa
Scand.J.Work Environ. & Health, **4(2)**, 156-162

Liu, SF *et al* (1988)
Detection of styrene oxide-DNA adducts in lymphocytes of a worker exposed to styrene.
In "Applications in Cancer Epidemiology and Prevention." IARC Scientific Publications. 89, 217-222.

Lof, A, Gullstrand, E and Nordqvist, MN (1983)
Tissue distribution of styrene, styrene glycol and more polar styrene metabolites in the mouse.
Scand.J.Work.Environ. Health, **9** 419-430.

Lof, A *et al* (1984)
Occurrence of styrene-7,8-oxide and styrene glycol in mouse after the administration of styrene.
Scand J. work environ. Health. **10**, 179-187.

Lof, A, Lundgren, E and Nordqvist, MB (1986a)
Kinetics of styrene in workers from a plastics industry after controlled exposure: a comparison with subjects not previously exposed.
British J. Ind. Med. **43**, 537-543.

Lof, A *et al* (1986b)
Biological monitoring of styrene metabolites in blood.
Scand J. Work Environ. Health **12**, 70-74.

Lof, A and Johanson, G (1993)
Dose-dependent kinetics of inhaled styrene in man.
IARC Scientific Publications. 127, 89-99.

Lomax RB, Ridgway P and Meldrum M (2004).
Does occupational exposure to organic solvents affect colour discimination?
Toxicol. Rev. **23(2)**, 91-121.

Loprieno N *et al* (1976)
Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide. Mutation Research 40, 317-324.

Loprieno, N *et al* (1978)
Mutagenicity of industrial compounds. VII. Styrene and styrene oxide. 11. Point mutations, chromosome aberrations and DNA repair induction analysis.
Scand J Work Environ Health. **4(suppl 2);** 169-78.

Loquet G, Campo P and Lataye R (1999)
Comparison of toluene-induced and styrene-induced hearing loss.
Neurotoxicol Teratol. **21;** 689-697.

Lorimer, WV et al (1976)
Clinical studies of styrene workers. Initial findings.
Environ Health Perspect. **17;** 171-81.

Lorimer, WV, Lilis, R, Fischbein, A et al (1978)
Health status of styrene-polystyrene polymerisation workers.
Scand J Work Environ Health. **4(suppl. 2)**; 220-226.

Loquet G, Campo P, Lataye R, Cossec B, Bonnet P (2000).
Combined effects of exposure to styrene and ethanol on the auditory function in the rat.
Hearing Research, **148**, 173-180.

Loughlin, JE, Rothman, KJ and Dreyer, NA (1999)
Lymphatic and haematopoietic cancer mortality in a population attending school adjacent to styrene-butadiene facilities, 1963-1993.
J Epidemiol community Health. **53**; 283-287.

Luderer, U, Tornero-Velez, R, Shay, T, Rappaport, S, Heyer, N, and Echeverria, D (2004)
Temporal association between serum prolactin concentration and exposure to styrene.
Occup Environ Med, **61**, 325-333.

Lutz, WK (1992)
Investigation of the adduct formation between styrene or styrene-7,8-oxide and deoxyribonucleic acid (DNA) in rats, mice and *in vitro*.
Technical Report 52, ECETOC.

Lutz, WK (1998)
The role of DNA adduct formation of styrene-7,8-oxide for tumour induction in the mouse lung by styrene (phase 1)
Unpublished draft report for the Styrene Steering Committee.

Lyman, WJ, Reehl, WF and Rosenblatt, DH (1982)
Handbook of Chemical Property Estimation Methods. McGraw-Hill, New York.

Macaluso, M, Larson, R, Delzell, E *et al* (1996)
Leukaemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry.
Toxicol. **113;** 1990-202.

MacFarlane, IA, Wilkinson, R and Harrington, JM (1984)
Severe postural hypotension following home canoe construction from polyester resins.
Postgraduate Medical Journal. **60;** 497-498.

Mackay, CJ and Kelman, GR (1986)
Choice reaction time in workers exposed to styrene vapour.
Human Toxicol. **5;** 85-89.

Mackay D, Shiu, WY and Ma, KC (1993)
Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals: Volume 3, Volatile organic chemicals. Lewis Publishers, Michigan.

MAFF (1983)
Survey of styrene levels in food contact materials and in food. Food Surveillance Paper No. 11.
Ministry of Agriculture, Fisheries and Food, UK. HMSO London.

MAFF (1994)
Food Surveillance Information Sheet Number 38.
Ministry of Agriculture, Fisheries and Food, UK. HMSO, London.

MAFF (1997)
National Food Survey 1996.
Ministry of Agriculture, Fisheries and Food, UK. HMSO, London.

MAFF (1999)
Food Surveillance Information Sheet Number 189. November 1999, MAFF UK – Total diet study: styrene.
Ministry of Agriculture, Fisheries and Food, UK. HMSO, London.
http://www.foodstandards.gov.uk/maff/archive/food/infsheet/1999/no189/189styr.htm.

Mahler, JF, Price, HC, O'Connor, TW *et al* (1999) Characterisation of hepatocellular resistance and susceptibility to styrene toxicity in B6C3F$_1$ mice.
Toxicological Sciences, **48**, 123-133.

Maki-Paakkanen, J (1987)
Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene.
Mutation Research. **189**, 399-406.

Maki-Paakkanen, J *et al* (1991)
Single-strand breaks, chromosome aberrations, sister-chromatid exchanges and micronuclei in blood lymphocytes of workers exposed to styrene during the production of reinforced plastics.
Environmental and Molecular Mutagenesis, **17**, 27-31.

Makitie, A (1997)
The ototoxic effect of styrene and its interaction with noise. Academic dissertation for PhD. Medical Faculty of the University of Helsinki. Finland.

Maltoni, C, Ciliberti, A and Carretti, D (1982)
Experimental contributions in identifying brain potential carcinogens in the petrochemical industry.
Ann. N.Y. Academy Sciences. **381**, 216-249.

Manini, P, De Palma, G, Mozzoni, P, Andreoli, R, Poli, D, Bergamaschi, E, Franchini, I, Mutti, A (2002).
GSTM1 polymorphism and styrene metabolism: insights from an acute accidental exposure.
Toxicology Letters, **134**, 201-208.

Manini, P, Buzio, L, Andreoli, R, Goldoni, M, Bergamaschi, E, Jakubowski, M, Vodicka, P, Hirvonen, A, Mutti, A (2003).
Assessment of biotransformation of the arene moiety of styrene in volunteers and occupationally exposed workers.
Toxicology and Applied Pharmacology, **189**, 160-169.

Mansour M, Moza PN, Barlas H, Parlar H (1985)
Ein beitrag zur photostabilität organischer umweltchemikalien in gegenwart von wasserstoffperoxid in aquatischen systemen.
Chemosphere, **14(10)**, 1469-1474.

Marczynski, B, Rozynek, P, Elliehausen, H-J *et al* (1997)
Detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in white blood cells of workers occupationally exposed to styrene.
Archives of Toxicology, **71**, 496-500.

Matanoski, GM *et al* (1982)
Mortality of workers in the styrene-butadiene rubber polymer manufacturing industry. John Hopkins University: Baltimore, USA (prepared under contract to the International Institute of Synthetic Rubber Products, Inc).

Matanoski, GM and Schwartz, L (1987)
Mortality of workers in styrene-butadiene polymer production.
Journal of Occupational Medicine. **29**, 675-680.

Matanoski, GM *et al* (1990)
Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982)
Environmental Health Perspectives **86**, 107-117.

Matanoski, GM (1993)
Cancer epidemiology among styrene-butadiene rubber workers.
In: Sorsa *et al* (eds). Butadiene and styrene: Assessment of health hazards. IARC Scientific Publications. No 127 IARC: Lyon, France. 363-374.

Matanoski, G, Elliott, E, Tao, X *et al* (1997)
Lymphohaematopoietic cancers and butadiene and styrene exposure in synthetic rubber manufacture.
Annals of the New York Academy of Sciences, **837**, 157-169.

Matanoski, G, Tao, X (2003).
Styrene exposure and ischaemic heart disease: a case-cohort study.
American Journal of Epidemiology, **158 (10)**, 988-995.

Matikainen, E *et al* (1993)
Nervous system effects of occupational exposure to styrene: A clinical and neurophysiological study.
Environmental Research. **61**, 84-92.

Matsuoki, A, Hayashi, M and Ishidate, M (1979)
Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*.
Mutation Research **66**, 277-90.

Mattson VR *et al* (1976)

Acute toxicity of selected organic compounds to fathead minnows, US EPA/600/3-76/097. Environmental Research Lab, Duluth, Minnesota.

Maurissen JPJ, Marable BR, Andrus AK and Stebbins KE (2003)
Factors affecting grip strength testing.
Neurotoxicol Teratol, **25**, 543-553.

McClellan, RO (ed) (1994)
Styrene toxicology and epidemiology.
Critical Reviews in Toxicology. **24/Supplement**, CRC Press.

McDonald, AD *et al* (1988)
Spontaneous abortion in women employed in plastics manufacture.
American Journal of Industrial Medicine **14**, 9-14.

McDougal, HN *et al* (1990)
Dermal absorption of organic chemical vapours in rats and humans.
Fund. Appl. Toxicol. **14**, 299-308.

McKay RT, Lemasterss GK, Elia VJ (1982)
Ambient air styrene levels in communities near reinforced plastic processors.
Environmental Pollution (Series B), **4**, 135-141.

McLellan, RO (1994)
Critical Reviews in Toxicology, **Vol 24 (supplement 1994)** – styrene. CRC press, USA.

McMichael, AJ, Spirtas, R, Kupper, LL (1974)
An epidemiologic study of mortality within a cohort of rubber workers, 1964-72.
J. Occup. Med. **16**, 458-464.

McMichael, AJ *et al* (1975)
Solvent exposure and leukaemia among rubber workers: an epidemiologic study.
J. Occup. Med. **17**; 234-239.

McMichael, AJ *et al* (1976)
Mortality among rubber workers: relationship to specific jobs.
J. Occup. Med. **18**; 178-185.

Meinhardt, TJ, Young, RJ, Hartle, RW (1978)
Epidemiologic investigations of styrene0butadiene rubber production and reinforced plastics production.
Scand. J. work Environ. Health. **4(suppl 2)**, 240-246.

Meinhardt, TJ *et al* (1982)
Environmental epidemiological investigation of the styrene-butadiene rubber industry.
Scand. J. Work Environ. Health. **8**, 250-259.

Mendrala, AL *et al* (1993)
In vitro kinetics of styrene and styrene oxide metabolism in rat, mouse and human.
Arch Toxicol. **67**, 18-27.

The Merck Index (1989)
The Merck Index, 11th edition, 1989.

Meretoja, T, Vainio, H and Jarventaus, H (1978a)

Clastogenic effects of styrene exposure on bone marrow cells of rate
Toxicology Letters **1**, 315-318.

Meretoja, T et al (1978b)
Chromosome aberrations in lymphocytes of workers exposed to styrene.
Scand. J. work Environ. Health. **4, suppl.2**, 259-264.

Meretoja, T, Vainio, H and Harkonen, H (1977)
Occupational styrene exposure and chromosomal aberrations>
Mutation Research. **56**, 193-197.

Mergler, D *et al* (1992)
Neurotoxic effects among styrene-exposed workers II Neurophysiological and behavioural changes.
Travail et sante 8, S16-S21.

Mergler, D, Huel, G, Belanger, S *et al* (1996)
Surveillance of early neurotoxic dysfunction.
NeuroToxicology **17 (3-4)** 803-812.

Migliore, L, Naccarati, A, Zanello, A, Scarpato, R, Bramanti, L, and Mariani M (2002)Assessment of sperm DNA integrity in workers exposed to styrene.
Human Reproduction, **17(11)**, 2912-2918.
Miller RR, Newhook R and Poole A (1994)
Styrene production, sue and human exposure. In: Styrene Toxicology and Epidemiology, ed RO McClellan. *Critical Reviews In Toxicology*, **24, Supplement**, CRC Press 1994.

Milvy, P and Garro, AJ (1976)
Mutagenic activity of styrene oxide (1,2-epoxyethylbenzene), a presumed styrene metabolite.
Mutation Research. **40**, 15-18.

Moller, C *et al* (1990)
Otoneurological findings in workers exposed to styrene
Scand. J. Work Environ. Health. **16**, 189-194.

Moller, P, Knudsen, LE, Loft, S, and Wallin, H (2000)
The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors.
Cancer Epidemiol. Biomarkers Prev. **9(10)**, 1005-1015.

Morata, TC, Johnson, AC, Nylen, P, Svensson, EB, Cheng, J, Krieg, EF, Lindblad, AC, Ernstgard L, Franks, J (2002)
Audiometric findings in workers exposed to low levels of styrene and noise.
J Occup Environ Med, **44**, 806-814.

Morgan, DL *et al* (1993a)
Styrene inhalation toxicity studies in mice. I. Hepatotoxicity in $B6C3F_1$ mice.
Fund. Appl. Toxicol. **20**, 325-335.

Morgan, DL *et al* (1993b)
Styrene inhalation toxicity studies in mice. II Sex differences in susceptibility of $B6V3F_1$ mice.
Fund. Appl. Toxicol. **21**, 317-325.

Morgan, DL, *et al* (1993c)
Styrene inhalation toxicity studies in mice. III Strain differences in susceptibility.
Fund. And Appl. Toxicology. **21**, 326-333.

Morgan, DL, Mahler, JJ, Moorman, P, *et al* (1995)
Comparison of styrene hepatotoxicity in B6C3F1 and Swiss mice.
Fundamental and Applied Toxicology **27**, 217-222.

Morioka, I, Kuroda, M, Myashiti, K *et al* (1999)
Evaluation of organic solvent ototoxicity.
Arch Env Health **54(5)**, 341-346.

Morris, JB (1999)
Uptake of styrene in the upper respiratory tract of the CD mouse and Sprague-Dawley rat.
Unpublished study report.

Morris, JB (2000)
Uptake of styrene in the upper respiratory tract of the CD mouse and Sprague-Dawley rat.
Toxicological Sciences. **54**, 222-228.

Moscato, G *et al* (1990)
Occupational asthma and rhinitis due to styrene.
Clinical and Experimental Allergy. **20**, 45.

Muijser, H, Hoogendijk, EMG and Hooisma, J (1988)
The effects of occupational exposure to styrene on high-frequency hearing thresholds.
Toxicology. **49**, 331-340.

Murata, K, Araki, S and Jokoyama, K (1991)
Assessment of the peripheral, central and autonomic nervous system function in styrene workers.
American J. Ind. Med. **20**, 775-784.

Murray, FJ (1978)
Teratologic evaluation of styrene given to rats and rabbits by inhalation or by gavage.
Toxicology. **11**, 335-343.

Mutti, A *et al* (1984)
Regional alterations of brain catecholamines by styrene exposure in rabbits.
Arch. Toxicol. **55**, 173 – 177.

Mutti, A *et al* (1984a)
Neuroendocrine effects of styrene on occupationally exposed workers.
Scand. J. Work Environ. Health. **10**, 225-228.

Mutti, A *et al* (1984b)
Exposure-effect and exposure-response relationships between occupational exposure to styrene and neuropsychological functions.
Am J Ind Med. **5;** 275-286.

Mutti, A *et al* (1992)
Lymphocytic subsets in workers occupationally exposed to styrene.
Med. Lav. **83**, 167-177.

Mutti, A, Coccini, T, Alinovi, R, *et al* (1999)

Exposure to hydrocarbons and renal disease: An experimental animal model.
Renal Failure. **21**; 369-385.

Muttray, A, Jung, D and Konietzko, J (1993)
Subclinical impairment of colour vision among workers exposed to styrene.
Brit J Ind Med. **50**; 765-768.

Muttray, A, Unger, C, Jung, D, Konietzko, J (1998a)
[Acquired dyschromatopsia in the workplace. Part I: Diagnosis and reference values.] *Arbeitsmed Sozialmed Umweltmed* **33**, 144-152 [Article in German; HSE translation 16467B].

Muttray, A, Wolters, V, Wolff, U, Konietzko, J (1998b)
[Acquired dyschromatopsia in the workplace. Part II: Studies on suitability for work of acquired and congenital dyschromatopsia.]
Arbeitsmed Sozialmed Umweltmed **33**, 183-189 [Article in German: HSE translation 16468B]

Muttray, A, Wolters, V, Jung, D, Konietzko, J (1999)
Effects of high doses of toluene on color vision.
Neurotoxicol Teratol, **21**, 41-5.

Naccarati, A, Zanello, A, Landi, S, Consigli, R, and Migliore, L (2003)
Sperm-FISH analysis and human monitoring: a study on workers occupationally exposed to styrene.
Mutation Research, **537**; 131-140.

Nakajima, T, Wang, R-S, Elovaara, E, *et al* (1994a)
CYP2C11 and CYP2B1 are major cytochrome P450 forms involved in styrene oxidation in liver and lung microsomes from untreated rats, respectively.
Biochem Pharmacol. **48**; 637-642.

Nakajima, T, Elovaara, E, Gonzalez, FJ, *et al* (1994b)
Styrene metabolism by cDNA-expresed human hepatic and pulmonary cytochromes P450.
Chem Res Toxicol. **7**; 891-896.

NCI (1979)
Bioassay of styrene for possible carcinogenicity.
Carcinogenesis Testing Program, National Cancer Institute, Bethesda, Maryland.
Report No. NIH 79-1741.

Nedelcheva, V (1996)
Interaction of styrene and ethylmethylketone in the induction of cytochrome P450 enzymes in rat lung, kidney and liver after separate and combined inhalation exposures.
Centre Eur J Publ Hlth. **4**; 115-118.

Neshkov, NS and Nosko, AM (1976)
Effect of toxic components of the fibre glass-reinforced plastics on the higher nervous activity and sexual function of males.
Gig Tr. **12**; 92-94.

Nicholson, WJ, Selikoff, IJ and Seidman, H (1978)
Mortality experience of styrene-polystyrene polymerisation workers. Initial Findings.
Scand J Work Environ Health. **4 (suppl. 2)**; 247-252.

Niklasson, M *et al* (1993)

Effects of toluene, styrene, trichloroethylene and trichloroethane on the vestibulo- and opto-oculo motor system in rats
Neurotoxicol Teratol. **15**; 327-334

NIOSH (1983)
National Institute for Occupational Safety and Health. Occupational exposure to styrene: Criteria for recommended standard. NIOSH 83-119, p17.

Nobuhara, Y, Hirano, S, Azuma, Y *et al* (1999)
Biological evaluation of styrene oligomers for endocrine-disrupting effects.
J Food Hygiene Soc Japan. **40**; 36-45.

Nordenson, I and Beckman, L (1984)
Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene.
Human Heredity. **34**; 178-182.

Nordqvist, MB *et al* (1985)
Covalent binding of styrene and styrene-7,8-oxide to plasma proteins, haemoglobin and DNA in the mouse.
Chem Biol Interactions. **55**; 63-73.

Norppa, H, Sorsa, M and Vainio, H (1980a)
Chromosomal aberrations in bone marrow of Chinese hamsters exposed to styrene and ethanol.
Toxicology Letters. **5**; 241-244.

Norppa, H, Sorsa, M, Pfafflli, P and Vainio, H (1980b)
Styrene and styrene induce SCEs and are metabolised in human lymphocyte cultures
Carcinogenesis, **1**; 357-361.

Norppa, H (1981)
Styrene and vinyltoluene induce micronuclei in mouse bone marrow.
Toxicology Letters. **8**; 247-251.

Norppa, H, Sorsa, M and Vainio, H (1981)
Commentary: Chromosome aberrations in lymphocytes of workers exposed to styrene.
American Journal of Industrial Medicine, **2**; 299-304.

Norppa, H, Tursi, F and Einisto, P (1983a)
Erythrocytes as a metabolic activation system in mutagenicity tests.
Mutagenes et Toxicologie Genetique Inserm Paris. 35-50.

Norppa, H, Vainio, H and Sorsa, M (1983b)
Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes.
Cancer Research. **43**; 3579-3582.

Norppa, H and Vainio, H (1983a)
Genetic toxicity of styrene and some of its derivatives.
Scand J. Work Environ, Health. **9**; 108-114.

Norpa, H and Vainio, H (1983b)
Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes.
Mutation Research. **116**; 379-387.

Norppa, H and Tursi, F (1984)
Erythrocyte-mediated metabolic activation detected by SCE.
In "Sister Chromatid Exchanges. 25 Years of Experimental Research. Part B Genetic Toxicology and Human Studies". (Rice, R.R. and Hollaender, A. Eds.)

Norrpa, H, Trusi, F and Einisto, P (1985)
Erythrocytes as a metabolic activation system in mutagenicity tests.
In: P. Janiaud, D. Averbeck and E. Moustacchi (Eds), Mutageneese et toxicologie genetigue, pp 35-48.

Norrpa, H, Jarventaus, H, Kubiak, R et al (1991)
Chromosome aberration, sister chromatin exchanges, and micronuclei in blood lymphocytes of Finnish reinforced plastics workers exposed to styrene.
Environ. Molec. Mutagen. **17(19)** 56.[Abstract No. 196]

Norstrom, A *et al* (1992)
Determination of N-acetyl-S-(2-phenyl-2-hydroxyethyl) cysteine in human urine after experimental exposure to styrene.
Chemosphere. **24**; 1553-1561.

Nylen P, Hansson AC, Hagman M, Hoglund G and Linder G (1987)
Inhalation exposure to toluene: effect of daily dose on auditory sensitivity and nerve conduction velocity in rats.
Proccedings of the XXII International Congress on Occupational Health, Sydney, Australia, Sept 27-Oct 2, Poster 24.6.

Odkvist, LM *et al* (1982)
Vestibulo-oculomotor disturbances in humans exposed to styrene.
Acta Otolaryngol. **94**; 487-493.

Ogata M, Fujisawa K, Ogino Y, Mano E (1984)
Partition coefficient as a measure of bioconcentration potential of crude oil compounds in fish and shellfish.
Bulletin of Environmental Contamination and Toxicology, **33**; 561-567.

Ohashi, Y, Nakai, Y, Ikeoda, H *et al* (1985)
Electron microscopic study of the respiratory toxicity of styrene Osaka City Med. J., 31, 11.

Ohashi, Y, Nakai, Y, Ikeoda, H *et al* (1986)
Degeneration and regeneration of respiratory mucosa of rats exposed to styrene
Toxicol. Appl. Pharmacol., **10**; 405.

Ohno, K, Azuma, Y, Nakano, S, Kobayashi, T, Hirano, S, Nobuhara, Y, Yamada, T (2001).
Assessment of styrene oligomers eluted from polystyrene-made food containers for estrogenic effects in *in vitro* assays.
Food and Chemical Toxicology, **39**, 1233-1241.

Okun, AH *et al* (1985)
Mortality patterns among styrene-exposed boatbuilders.
American J. Ind. Med. **8**; 193-205.

Oltramare, M *et al* (1974)
Toxicology of styrene monomer, studies in man.
Editions Medecine et Hygiene, Geneva.

Oston R (1987)
Purgeable organics in Great Lakes raw and treated water.
Intern. J. Environ. Anal. Chem., **31**; 41-53.

Ott, MG *et al* (1980)
A mortality survey of employees engaged in the development or manufacture of styrene-based products.
Journal of Occupational Medicine. **22**; no 7, 445-460.

Otteneder M, Eder E, and Lutz WK (1999)
^{32}P-Postlabelling analysis of DNA adducts of styrene 7,8-oxide at the O^6 position of guanine.
Chem. Res. Toxicol. **12**, 93-99.

Otteneder, M, Lutz, U, Lutz, W (2002).
DNA adducts of styrene-7,8-oxide in target and non-target organs for tumour induction in rat and mouse after repeated inhalation exposure to styrene.
Mutation Research, **500**, 111-116.

Pagano, D, Yagen, B, Hernandez, O, Bend, J, Zeiger, E (1982).
Mutagenicity of (R) and (S) styrene 7,8-oxide and the intermediary mercapturic acid metabolites formed from styrene 7,8-oxide.
Environmental Mutagenesis, **4**, 575-584.

Pantarotto, C, Fanellii, R, Bidoli, F *et al* (1978)
Arene oxides in styrene metabolism, a new perspective in styrene toxicity?
Scand.J. Work. Environ & Health, **4 (suppl. 2)**; 67-77.

Parent, M-E, Hua, Y, Siemiatycki, J (2000).
Occupational risk factors for renal cell carcinoma in Montreal.
American Journal of Industrial Medicine, **38**, 609-618.

Parrki, MG (1978)
The role of glutathione in the toxicity of styrene.
Scand. J. Environ. & Work, **4(suppl. 2)**, 47-52

Pauwells, W, Vodiceka, P, Severi, M *et al* (1996)
Adduct formation on DNA and haemoglobin in mice intraperitoneally administered with styrene.
Carcinogenesis, **17(12)**, 2673-2680

Pekari, K *et al* (1993)
Biological monitoring of exposure to styrene-assessment of different approaches.
Journal of Occupational Medicine and Toxicology. **2**, 115-126.

Pellizzari ED, Hartwell TD, Perritt RL, Sparacino CM, Sheldon LS, Zelon HS, Whitmore R W, Breen JJ, Wallace L (1986)
Comparison of indoor and outdoor residential levels of volatile organic chemicals in 5 US geographical areas.
Environment International, **12**, 619-623.

Penttila, M, Sorsa, M and Vainio, H (1980)
Inability of styrene to induce nondisjuction in Drosophila or a positive micronucleus test in the Chinese hamster.
Toxicology Letters. **6**, 119-123.

Pero, RW, Bryngelsson, T, Hogstedt, B and Akesson, B (1982)
Occupational and *in vitro* exposure to styrene assessed by unscheduled DNA synthesis in resting human lymphocytes.
Carcinogenesis **3**, 681-685.

Petreas, MX, Woodlee, J, Becker, CE and Rappaport, SM (1995)
Retention of styrene following controlled exposure to constant and fluctuating air concentrations
International Archives of Occupational and Environmental Health, **67**, 27-34.

Pfaffli, P, Hesso, A, Vainio, H and Hyvonen, M (1981)
4-Vinylphenol excretion suggestive of arene oxide formation in workers occupationally exposed to styrene.
Toxicology and Applied Pharmacology, **60**, 85-90.

Phillippe, R *et al* (1971)
Measurement of exposure of workers to styrene by assay of urinary metabolites, mandelic and phenylglyoxylic acid. II. Application to workers manufacturing polyesters.
Arch. Mal. Prof. **35**, 631-640.

Pickering QH and Henderson CH (1966)
Acute toxicity of some important petrochemicals to fish.
Journal WPCF, **38(9)**, 1419-1429.

Piece, CH, Dilis, RL, Silvey, GW and Kalman, DA (1996)
Partition coefficients between human blood or adipose tissue and air for aromatic solvents.
Scand. J. Work. Environ. Health. **22**, 112-118.

Pierce, CH, Becker, CE, Tozer, TN, Owen, DJ, and So, Y (1998)
Modelling the acute neurotoxicity of styrene.
J. Occup. Environ. Med. **49**, 230-240.

Plopper, CG,Mariassy, AT and Hill LH (1980)
Ultrastructure of the nonciliated bronvhiolar epithelial (Clara) cell of mammalian lung: I. A comparison of rabbit, guinea pig, rat, hamster and mouse.
Experimental Lung Research. **1**:139-154.

Plotnick, HB and Weigel, WW (1979)
tissue distribution and excretion of 14C-styrene in male and female rats.
Res. Commun. Chem. Pathol. Pharmacol. **24**, 515-524.

Pohlova, H and Sram, RJ (1985a)
Cytogenetic analysis of peripheral blood lymphocytes of workers occupationally exposed to styrene.
Journal of Hygiene, Epidemiology, Microbiology and Immunology. **28**, 155-161.

Pohlova, H, rossner, P and Sram, RJ (1985b)
Cytogenetic analysis of human peripheral blood lymphocytes in culture exposed *in vitro* to styrene and styrene oxide.
Journal of Hygiene, Epidemiology, Microbiology and Immunology. **29**, 269-274.

Poncelet, F, de Meester, C, Duverger-van Bogaert, M *et al* (1980)
Influence of experimental factors on the mutagenicity of vinylic monomers.
Arch. Toxicol **4**, 63-66.

Ponomarkov, V and Tomatis, L (1978)
Effects of long-term oral administration of styrene to mice and rats.
Scand. J. work. Environ. Health. **4, suppl. 2**, 127-135.

Popler, A, Ciharova, M, Salandova, J and Skutilova, I (2989)
Monitoring of exposure to styrene. II. Evaluation of the results of a cytogenetic analysis of the peripheral lymphocytes.
Pracov Lek., **41(2)**, 51-56.

Pouyatos B, Morel G, Lambert-Xolin A-M, Maguin K and Campo P (2004)
Consequences of noise- or styrene-induced cochlear damages on glutamate decarboxylase levels in the rat inferior colliculs.
Hearing Research, **189**, 83-91.

Pratt-Johnson, JA (1964)
Retrobulbar neuritis following exposure to vinyl benzene (Styrene).
Can. Med. Assoc. J. **90**, 975-977.

Preston, RJ and Abernethy, DJ (1993)
Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation.
IARC Scientific Publications No. **127** 225-233.

Price KS, Waggy GT, Conway RA (1974)
Brine shrimp bioassay and seawater BPD of petrochemicals.
J. Water Poll. Cont. Fed., **46(1)**, 63-77.Pryor, GT, Rebert, CS and Howd, RA (1987)
Hearing loss in rats caused by inhalation of mixed xylenes and styrene.
Journal of Applied Toxicology **7**, 55-61.

Pryor GT, Rebert CS, Dickinson J and Feeney EM (1984)
Factors affecting toluene-induced ototoxicity in rats.
Neurobehavioral Toxicol. Teratol. **6**: 223-238.

Quast, JC, Humiston, CG and Kalvins, RV (1979)
Results of a toxicity study of monomeric styrene administered to Beagle dogs by oral intubation for 19 months. The Dow Chemical Company.
Cited in ECETOC Technical report No. 52.

Quereshi AA, Flood KW, Thompson SR, Janhurst SM, Innis CS and Rokosh DA (1982)
Comparison of a luminescent bacterial test with other bioassays for determining toxicity of pure compounds and complex effluents.
In: Aquatic Toxicology and Hazard Assessment, 5th conference (Pearson JG, Foster R B and Bishop W E eds). ASTM STP766 Am. Soc. For testing and Materials, Philadelphia, p179-195.

Rarul'ye, N (1974)
The problem of the embryotrophic action of styrol.
Gig. Sanit. 85-86.

Ramsey, JC and Young, JD (1978)
Pharmacokinetics of inhaled styrene in rats and humans.
Scand. J. Work. Environ. Health. **4**, 84-91.

Ramsey, JC *et al* (1980)
Pharmacokinetics of inhaled styrene in Human volunteers.

Toxicol. Appl. Pharmacol. **53**, 54-63.

Rebert CS, Boyes WK, Pryor GT *et al* (1993) Combined effects of solvents on the rat's auditory system: styrene and trichloroethylene.
Int. J Psycholo. **14**, 49-59

Reigel (1974)
Reigel's Handbook of Industrial Chemistry, 7th Edition. Edited by J A Kent. Van Nostrand Reinhold, New York. USA

Reinders ME (1984)
Handbook of Emission Factors, Part 2: Industrial Sources. Ministry of Housing, Physical Planning and Environment, The Netherlands.

RTI (1992)
Research Triangle Institute. Evaluation of exposure to volatile organics offgassing from new carpets. Final Report. RTI Report Number 94U-4479-001/12-F. Available from Environmental Criteria and Assessment Office, US EPA, Research Triangle Park, NC 27709.

Riihimaki, V and Pfaffli, P (1978)
Percutaneous absorption of solvent vapours in man.
Scand J Work Environ Health. **4;** 73-85.

RIVM, VROM, WVC (1994)
Uniform System for the evaluation of Substances (USES), version 1.0 National Institute of Public Health and Environmental Protection (RIVM), Ministry of Housing, Spatial Planning and the Environment (VROM), Ministry of Welfare, Health and Cultural Affairs (WVC). The Hague, Ministry of Housing, Spatial Planning and the Environment. Distribution No. 11144/150.

Robinson, DR, Goodall, K, Albertini, RJ, O'Neill, JP, finette, B, Sala-trepat, M, Moustacchi, E, Tates, AD, Beare, DM, Green, MHL and Cole, J (1994)
An analysis of *in vivo* hprt mutant frequency in circulating T-lymphocytes in the normal human population: a comparison of four data sets.
Mutation Research. 313; 227-247.

Romanelli A, Falzoi M, Mutti A, Bergamaschi E and Franchini I (1986)
Effects of some monocyclic aromatic solvents and their metabolites on brain dopamine in rabbits.
J Appl Toxicol. **6;** 431-435.

Rosen, I *et al* (1978)
Neurophysiological observations after chronic styrene exposure.
Scand J Work Environ Health. **4** (suppl 2); 184-194.

Rosengren LE and Haglid KG (1989)
Longterm neurotoxicity of styrene. A quantitative study of glial fibrillary acidic protein (GFA) and S-100.
Brit J Ind Med. **46;** 316-320.

Roycroft, JH, Mast, TJ, Ragan, HA *et al* (1992)
Toxicological effects of inhalation exposure to styrene in rats and mice [Abstract].
Toxicologist **12;** 397.

Ruder, A, Ward, E, Dong, M, Okun, A, Davis-King, K (2004).
Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update.
American Journal of Industrial Medicine, **45**, 165-176.

Runge, D.M., Stock, T.W., Lehmann, T., Taege, C., Bernauer, U., Stolz, D., Hofmann, S. and Foth, H. (2001).
Activation of Cytochrome P450 2E1 by ethanol in normal human bronchial epithelial cells. *Arch. Tox.* **75***,* 335-345*.*

Ryan, AJ (1976)
Hepatic and extrahepatic metabolism of ^{14}C-styrene oxide.
Environ health Perspect. **17;** 135-144.

Salomaa, S, Donner, M and Norppa, H (1985)
Inactivity of styrene in the mouse sperm morphology test.
Toxicology Letters. **24;** 151-155.

Savolainen H and Pfaffli P (1977)
Effects of chronic styrene inhalation exposure on rat brain protein metabolism.
Acta Neuropathologica. **40;** 237-241.

Sandell J (1978)
Effects of inhalation and cutaneous exposure to styrene on drug metabolising enzymes in the rat.
Res. Commun. Chem. Pathol. Pharmacol. **19**, 109-118.

Santos-Burgoa, C *et al* (2992)
Lymphohematopoietic cancer in styrene-butadiene polymerisation workers.
American Journal of Epidemiology. **136(7)**, 843-853.

Sarangapani, R, Teeguarden, J, Cruzan, G, Clewell, H, Andersen, M (2002).
Physiologically based pharmacokinetic modeling of styrene and styrene oxide respiratory-tract dosimetry in rodents and humans.
Inhalation Toxicology, **14**, 789-833.

Sass-Kortsak, AM, Corey, PN and Robinson, JMcD (1995)
An investigation of the association between exposure to styrene and hearing loss. Ann *Epidemiol*.**5**, 15-24.

Sass-Kortsak, AM, Bozek, PR, Corey, PN *et al* (1996)
Styrene and noise exposures and protective equipment use in the manufacturing of Fiberglas products in Canada.
Applied Occup and env Hygiene **11**(8), 1081-1086.

Sathiakumar, N, Delzell, E, Hovinga, M *et al* (1998)
Mortality from cancer and other causes of death among synthetic rubber workers.
Occupational Environmental Medicine, **55**, 230-235.

Savolainen, H and Vainio, H (1977)
Organ distribution and nervous system binding of styrene and styrene oxide.
Toxicology. **8**, 135-141.

Savalainen, H and Pfaffli, P (1977)
Effects of chronic styrene inhalation on rat brain protein metabolism

Acto. Neuropath, (Berl.) **40**, 237 – 241.

Savolainen, H and Pfaffli, P (1978)
accumulation of styrene monomer and neurochemical effects of long-term inhalation exposure in rats.
Scand. J. Work Environ. and Health, **4(suppl.2)**, 78-83.

Savolainen, H *et al* (1980)
Behavioural and gial effected
Acta Pharmcol Toxicol. **46**, 51 – 56.

Sbrana, I *et al* (1983)
Bone marrow cell chromosomal aberrations and styrene biotransformation in mice given styrene on a repeated oral schedule.
Chem. Biol. Interactions **45**, 349-357.

Schoenhuber, R and Gentilini, M (1989)
Influence of occupational styrene exposure on memory and attention.
Neurotoxicology and Teratology. **11**, 585-586.

Seeber, A, Blaszkewicz, M, Golka, K, Hallier, E, Kiesswetter, E, Schaper, M, van Thriel, C (2004).
Neurobehavioural effects of experimental exposures to low levels of styrene.
Toxicology Letters, **151**, 183-192.

Senma, M *et al* (1978)
Studies on the cutaneous sensitisation reaction of guinea pigs to purified aromatic chemicals.
Acta Dermatovener (Sockholm) **58**, 121-124.

Seidel, HK, Herkommer, J, Seitz, D *et al* (1990)
Haemopoietic stem cells in mice chronically exposed to styrene vapour.
Arch. Toxicol. **64**, 466-469.

Semple, S, Dick, F, Osborne, A *et al* (2000)
Impairment of colour vision in workers exposed to organic solvents.
Occup Env Med **57**, 582-587.

Seppalainen, AM (1978)
Neurotoxicity of styrene in occupational and experimental exposure.
Scand. J. Work. Environ. Health. **4**, 181-183.

Seppalainen, AM, and Harkonen, H (1974)
Neurotoxicity of styrene in humans.
Proc 2nd Int Congress Ind environ Neurol, Prague. 155-158.

Severi, M, Pauwels, W, Van Hummelen, P *et al* (1994)
Urinary mandelic acid and haemoglobin adducts in fibreglass-reinforced plastics workers exposed to styrene.
Scand. J. Work Environ. Health, **20**, 451-458.

Shamy, MY, Osman, HH, Kandeel, KM, et al. (2002)
DNA single strand breaks induced by low levels of occupational exposure to styrene: the gap between standards and reality.
J. Environ. Path. Toxicol. Oncol. **21**, 57-61.

Sharief, Y *et al* (1986)
Sister chromatid exchange and chromosome aberration analyses in mice after in vivo exposure to acrylonitrile, styrene or butadiene monomer.
Environmental Mutagenesis. **8**, 439-448.

Sheedy, JE (1997)
Styrene exposure and color vision.
SIRC Review **5**, 7-30

Shell International Chemical Co. Ltd., Unpublished data.Shigeta, S *et al* (1989)
Effects of postnatal low-levels of exposure to styrene on behaviour and development in rats.
The Journal of Toxicological Sciences. **14**, 279-286.

Shugaev, BB and Yaroslavi, B (1969)
Concentrations of hydrocarbons in tissues as a measure of toxicity.
Arch. Environ. Health. **18**, 878-882.

Sielicki M *et al* (1978)
Appl. Environ. Microbiol. **35**, 124-128.

Simula, AP and Priestly, BG (1992)
Species differences in the genotoxicity of cyclophosphamide and styrene in three *in vivo* assays.
Mutation Research. **271**, 49-58.

Singh HB *et al* (1984)
Reactivity/volatility classification of selected organic chemicals; existing data.
US-EPA-600/3-84-082, p190

Sinha, AK *et al* (1983)
Cytogenetic evaluation of bone marrow cells from rats exposed to styrene vapour for one year.
Fund. Appl. Toxicol. **3**, 95-98.

Sinsheimer, J, Chen, R, Das, S, Hooberman, B, Osorio, S, You, Z (1993).
The genotoxicity of enantiomeric aliphatic epoxides.
Mutation Reseach, **298**, 197-206.

Sjoborg, S *et al* (1982)
Contact allergy to styrene with cross reaction to vinyltoluene.
Contact Dermatitis. **8**, 207-208.

Sjoborg, S, Fregert, S and Trulsson, L (1984)
Contact allergy to styrene and related chemicals.
Contact Dermatitis. **10**, 94-96.

Ska, B, Vyskocil, A, Tardif, R, Carrier, G, Thuot, R, Muray, K and Viau, C (2003)
Effects of peak concentrations on the neurotoxicity of styrene in volunteers.
Human & Experimental Toxicology, **22**, 407-415.

Skoog, KO, Nilsson, SE (1981)
Changes in the c-wave of the electroretinogram and in the standing potential of the eye after small doses of toluene and styrene.
Acta Ophthalmol, **59**, 71-9.

Sliwinska-Kowalska, M, Zamyslowska-Szmytke, E, Szymczak, W, Kotylo, P, Fiszer, M, Wesolowski, W, Pawlaczyk-Luszczyska, M (2003).
Ototoxic effects of occupational exposure to styrene and co-exposure to styrene and noise.
American Journal of Occupational and Environmental Medicine. **45** (1), 15-24.

Solveig-Walles, SAS and Orsen, I (1983)
Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide.
Cancer Letters. **21**, 9-15.

Somorovska, M, Jahnova, E, Tulinska, J *et al* (1999)
Biomonitoring of occupational exposure to styrene in a plastics lamination plant.
Mut. Res. **428(1-2)**, 255-269.

Sorsa, M *et al* (1991)
Sister chromatid exchange and chromosome aberration analyses in mice after *in vivo* exposure to acrylonitrile, styrene or butadiene monomer.
Environmental Mutagenesis. **8**, 439-448.

Soto, AM, Sonnenschein, C, Chung, KL et al (1995)
The E-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants.
Environ. Health Perspectives, **103(suppl.7)**, 113-122.

Spencer, HC et al (1942)
The response of laboratory animals to monomeric styrene.
J. Ind. Hyg. Toxicol. **24**, 295-301.

Srivastava, SP, Das, M, Mushtaq, M et al (1982)
Hepatic effects of orally administered styrene in rats.
Journal of Applied Toxicology **2(4)**, 219-222.

Srivastava, S, Seth, PK and Srivastava, SP (1989)
Effect of styrene administration on rat testis.
Arch. Toxicol. **63**, 43-46.

Srivastava, S, Srivastava, SP and Seth, PK (1990)
Embryo/fetotoxicity of styrene in rats.
J. Environ. Biol. **11**, No. 1, 73-77.

Srivastava, S, Seth, PK and Srivastava, SP (1992a)
Biochemical and morphological studies in testes of rat offspring of mothers exposed to styrene during lactation.
Pharmacology and Toxicology. **70**, 314-316.

Srivastava, S, Seth, P, Srivastava, SP (1992b)
Effect of styrene on testicular enzymes of growing rat.
Indian Journal of Experimental Biology. **30**. 399-401.

Stergel, B et al (1990)
Haematological findings among styrene-exposed workers in the reinforced plastics industry.
Int. Arch. Occup. Environ. Health. **62**, 11-18.

Stetkarova, I et al (1993)

Somatosensory evoked potentials in workers exposed to toluene and styrene.
British J. Ind. Med. **50**, 520-527.

Stewart, RD et al (1968)
Human exposure to styrene vapour.
Arch. Environ. Health. **16**, 656-662.

Stoltz, DR and Withey, RJ (1977)
Mutagenicity testing of styrene and styrene epoxide in Salmonella typhimurium.
Bull. Environ. Contam. Toxicol. **17**, 739-742.

Stump F, Tejada S, Ray W, Dropkin D and Black F (1990)
The influence of ambient temperature on tailpipe emissions from 1985 to 1987 model year light-duty gasoline motor vehicles – II.
Atoms Environ, **24A(8)**, 2105-2112.

Sumner, SCJ, Asgharian, B, Moss, O, Cattley, RC and Fennell, TR (1995)
Correlating styrene metabolism and distribution with hepatotoxicity.
The toxicologist, **15**, 4.

Sumner, SCJ, Cattley, RC, Asgharian, B et al (1997)
Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6CEF$_1$ mice and CD-1 mice following single and repeated inhalation exposures.
Chemico-Biological Ineractions, **106**, 47-65.

Sumner, SCJ and Greenlee, WF (1999)
Metabolism of styrene in human volunteers.
Unpublished study report. Submitted to Styrene Information & Research Center by the Chemical Industry Institute of Toxicology.

Takao, T, Nanamiya, W, Nazarloo, H-P, Asaba, K, Hashimoto, K (2000).
Possible reproductive toxicity of styrene in peripubertal male mice.
Endocrine Journal. **47 (3)**, 343-347.

Taskinen, H *et al* (1989)
Spontaneous abortions and congenital malformations among wives of men occupationally exposed to organic solvents.
Scand. J. Work Environ. Health. **15**, 345-352.

Tates, AD *et al* (1994)
Measurement of frequencies of *HPRT* mutants, chromosomal aberrations, micronuclei, sister-chromatid exchanges and cells with high frequencies of SCEs in styrene/dichloromethane-exposed workers.
Mutation Research **313**, 249-262.

Teixeira, JP, Gaspar, J, Silva, S, Torres, J, *et al* (2004)
Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1.
Toxicology, **195**, 231-242.

Teramoto, K *et al* (1993)
Comparison of the neurotoxicity of several chemicals estimated by the peripheral nerve conduction velocity in rats.
Environmental Research **62**, 148-154.

Teramoto, K and Horiguchi, S (1979)
Absorption, distribution and elimination of styrene in man and experimental animals.
Arh.hig.rada.toksikol., **30(suppl.)**, 431-437.

Teramoto, K and Horiguchi, S (1981)
Distribution, elimination and retention of styrene in rats.
The Journal of Toxicological Sciences. **6**, 13-18.

Theiss, AM and Friedheim, M (1978)
Morbidity among persons employed in styrene production, polymerisation and processing plants.
Scand. J. Work Environ. & Health, **4(suppl.2)** 203-214.

Thiess, AM and Fleig, I (1978)
Chromosome investigations on workers exposed to styrene/polystyrene.
Journal of Occupational Medicine, **20(11)**, 747-749.

Thies, AM, Scwegler, H and Fleig, I (1980)
Chromosome investigations in lymphocytes of workers employed in areas in which styrene-containing unsaturated polyester resins are manufactured.
American Journal of Industrial Medicine. **1**, 205-210.

Tomanin, R, Ballarin, G, Bartolucci, GB *et al* (1992)
Chromosome aberrations and micronuclei in lymphocytes of workers exposed to low and medium levels of styrene.
International Archives of Occupational and Environmental Health. **64**, 209-215.

Triebig, G, Schaller, K-H and Valentin, H (1985)
Investigations on neurotoxicity of chemical substances in the workplace. VII.
Longitudinal study with determination of nerve conduction velocities in persons exposed to styrene.
Int. Arch. Occup. Environ. Health. **56**, 239-247.

Triebig, G *et al* (1989)
Clinical and neurobehavioural study of the acute and chronic neurotoxicity of styrene
British J. Ind. Med. **46**, 799-804.

Triebig, G, Stark, T, Ihrig, A, Dietz, MC (2001)
Intervention study on acquired color vision deficiencies in styrene-exposed workers.
J Occup Environ Med, **43**, 494-500.

Truchon, G, Gerin, M and Brodeur, J (1990)
Urinary excretion of mandelic, phenylglyoxylic, and specific mercapturic acids in rats exposed repeatedly by inhalation to various concentrations of styrene vapours.
Canadian Journal Physiol. Pharmacol. **68**, 556-561.

Truchon, G, Gegin, D, Lesage, J *et al* (1998)
Urinary excretion of thioethers related to styrene exposure.
Journal of Occupational Health, **40**, 350-355.

Truchon, G *et al* (1992)
Neurotoxic effects among styrene-exposed workers: I Environmental and biological monitoring of exposure.
Travail et sante 8, S11-S14.

Tsai, S-Y and Chen, J-D(1996)
Neurobehavioural effects of occupational exposure to low-level styrene.
Neurotoxicology and Teratology **18**, no 4, 463-469.

Tsuruta, H (1982)
Percutaneous absorption of organic solvents.
Industrial Health. **20**, 335-345.

Tuazon EC, Aray J, Atkinson R and Aschmann SM (1993)
Gas-phase reactions of 2-vinylpuridine and styrene with OH and NO_3 radicals and O_3.
Environ. Sci. Technol., **27**, 1832-1841.

Tursi, F, Samaia, M, Salmona, M and Belvedere, G (1983)
Styrene oxidation to styrene oxide in human erythrocytes is catalysed by oxhaemoglobin.
Experientia, **39**, 593-595.

Tynan P, Watts CD, Sowray A, Hammond I and Crathorne B (1990)
The Transport and fate of organic pollutants in rivers. Final report for the National Rivers Authority and the Scotland and Northern Ireland Forum for Environmental Research.
WRC Report No PRS 2402-M/1, August 1990.

US EPA (1994)
ECOSAR. A computer programme to estimate the toxicity of industrial chemicals based on structure-activity relationships. R D Clements and V Nabholz, Project No PB94 500485.

Vaghef, H, and Hellman, B (1998)
Detection of styrene and styrene oxide-induced DNA damage in various organs of mice using the comet assay.
Pharmacol. Toxicol. **83**, 69-74.

Vainio, H (1976)
A study on the mutagenic activity of styrene and styrene oxide.
Scand.J. Work. Environ. Health. **3**, 147-151.

Vainio, H, Hemminki, K and Elovaara, E (1977)
Toxicity of styrene and styrene oxide on chick embryos.
Toxicology, **8**, 319-325.

Vainio, H and Zitting, A (1978)
Interaction of styrene and acetone with drug biotransformation enzymes in rat liver.
Scand. J. Environ. & work, **4(suppl.2)**, 47-52.

Vainio, H, Jarvisalo, J and Taskinen, E (1979)
Adaptive changes caused by intermittent styrene inhalation on xenobiotic transformation.
Toxicology and Applied Pharmacology, **49**, 7-14.

Van Hummelen, P *et al* (1994)
Cytogenetic analysis of lymphocytes from fibreglass-reinforced plastics workers occupationally exposed to styrene.
Mutation Research. **310**, 157-165.

Varner SL and Breder CV (1964a)
Headspace sampling and gas chromatographic determination of styrene migration from food-contact polystyrene cups into beverages and food stimulants.
J. Assoc. Off. Anal. Chem., **64(5)**, 1122-1130.

Varner SL and Breder C (1964b)
Liquid chromatographic determination of residual styrene in polystyrene food packaging.
J. Assoc. Off. Anal. Chem., **64(3)**, 647-652.

Vergieva, T, Zaykov, K, and Palatov S (1979)
A study of the embryotoxic effects of styrene.
Khig Zdraveopazvane **22**, 39-43.

Verpalmke, AJW and Herber, RFM (1998)
Effects on the kidney of occupational exposure to styrene.
International Archives of Occupational and Environmental Health, **71**, 47-52.

Verscheuren K (1983)
Handbook of Environmental Data on Organic Chemicals. 2nd Edition. Van Nostrand Reinhold Company Inc., New York.

Vettori, M-V, Corradi, D, Coccini, T, Carta, A, Cavazzini, S, Manzo, L, Mutti, A (2000).
Styrene-induced changes in amacrine retinal cells: an experimental study in the rat.
Neurotoxicology, **21(4)**, 607-614.

Viau, C *et al* (1987)
Evaluation of the nephrotoxic potential of styrene in man and in rat.
Journal of Applied Toxicology. **7, no. 5**, 313-316.

Viaene, MK, Veulemans, H, and Masschelein, R (1998)
Experience with a vocabulary test for workers previously and still exposed to styrene.
Scand J Work Environ Health, **24(4)**, 308-311.

Viaene, MK, Pauwels, W, Veulemans, H, Roels, HA, and Masschelein, R (2001)
Neurobehavioural changes and persistence of complaints in workers exposed to styrene in a polyester boat building plant: influence of exposure characteristics and microsomal epoxide hydrolase phenotype.
Occup Environ Med, **58**, 103-112.

Vodicka, P, Vodickova, L and Hemminki, K (1993)
^{32}P-postlabelling of DNA adducts of styrene-exposed lamination workers.
Carcinogenesis. **14**, 2059-2061.

Vodicka, P, Vodickova, L, Trejbalova, K, Sram, RJ, and Hemminki K (1994)
Persistence of O^6-guanine DNA adducts in styrene exposed lamination workers determined by ^{32}P-postlabelling.
Carcinogenesis, **15(9)**, 1949-1953.

Vodicka, P, Blastlova, T, Vodickova, L, Peterkova, K, Lmbert, B, and Hemminki, K (1995)
Biomarkers of styrene exposure in lamination workers: levels of O^6-guanine DNA adducts, DNA strand breaks and mutation frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes.
Carcinogenesis. **16(7)**, 1473-1481.

Vodicka, P, Tvrdik, T, Osterman-Golkar, S et al (1999)
An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers.
Mutat Res. **445(2)**:205-224.

Vodicka, P, Koskinen, M, Vodickova, L, Stetino, R, Smerak, P, Barta, I, Hemminki, K (2001).

DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation.
Chemico-Biological Interactions, **137**, 213-227.

Vodicka, P, Stetino, R, Koskinen, M, et al (2002a)
New aspects in the biomonitoring of occupational exposure to styrene.
Int Arch Occup Environ Health, **75(suppl)**, S75-S85.

Vodicka, P, Koskinen, M, Arand, M, et al (2002b)
Spectrum of styrene-induced DNA adducts: the relationship to other biomarkers and prospects in human biomonitoring.
Mutation Res, **511**, 239-254.

Vodicka, P, Koskinen, M, Stetina, R, et al (2003)
The role of various biomarkers in the evaluation of styrene genotoxicity.
Cancer Detection and Prevention, **27**, 275-284.

Vogie, KM, Mantick, NA, and Carlson, GP (2004)
Metabolism and toxicity of the styrene metabolite 4-vinylphenol in CYP2E1 knockout mice.
J. Toxicol. Environ. Health, Part A, **67**, 145-152.

Wallace, L (1986)
Personal exposures, indoor and outdoor air concentrations, and exhaled breath concentrations of selected volatile organic compounds measured for 600 residents of new Jersey, North Dakota, North carolina and california.
Toxicol. and Environ. Chem., **12**, 215-236.

Wallace, LA and Pellizzari, Ed (1986)
Personal air exposures and breath concentrations of benzene and other volatile hydrocarbons for smokers and nonsmokers.
Toxicology Letters **35**, 113-116.

Wallace, LA *et al* (1987)
Emissions of volatile organic compounds from building materials and consumer products.
Atmospheric Environment. **21**, 385-393.

Walles, SAS *et al* (1993)
Exposure dependent increase in DNA single strand breaks in leucocytes from workers exposed to low concentrations of styrene.
British J. Ind. Med. **50**, 570-574.

Walles, SAS *et al* (1988)
Single-strand breaks in DNA of peripheral lymphocytes of styrene-exposed workers.
IARC Scientific publications 89, 223-226.

Wang Y-P (1998)
Effects of styrene exposure on middle latency auditory evoked potentials and glial cells in rat.
Hokkaido J Med Sci **73**, 157-170.

Wang Y, Saito T, Hosokawa T and Saito K (1998)
Alteration in hippocampal and cerebral expression of glial fibrillary acidic protein following styrene exposure in rat.
Neuropathology **18**, 289-294.

Watabe, T *et al* (1978a)

Metabolism and mutagenicity of styrene.
Scand. J. Work. Environ Health. **4, suppl 2**, 142-155.

Watabe, T, Isobe M, Yoshikawa, K and Takabatake, E (1978b)
Studies on metabolism and toxicity of styrene. II. Mutagenesis in Salmonella typhimurium by metabolic activation of styrene with 3-methlcholanthrene pretreated rat liver.
J.Pharm.Dyn. **1**, 301-309.

Watabe, T, Hiratsuka, A, Sone, T, Ishihama, T, Endoh, K (1984)
Hepatic microsomal oxidation of styrene to 4-hydroxystyrene 7,8-glycol via 4-hydroxystyrene and its 7,8-oxide as short-lived intermediates.
Biochem. Pharmacol. **33**, 3101-3103.

Watanabe, T, Endo, A, Sato, K *et al* (1981)
Mutagenic potential of styrene in man.
Industrial Health. **19**, 37-45.

Watanabe, T, Endo, A, Kumai, M and Ikeda, M (1983)
Chromosome aberrations and sister chromatid exchanges in styrene-exposed workers with reference to their smoking habits.
Environmental Mutagenesis. **5**, 299-309.

Watts, CD and Moore, K (1989)
The transport and fate of organic pollutants in rivers.
WRC Report PRU 1869-M. WRc Environment, Medmenham, Bucks. UK.

Welp, E, Partanen, T, Kogevinas, M *et al* (1996a)
Exposure to styrene and mortality from non-malignant respiratory diseases.
Occupational and Environmental Medicine. **53**, 499-501.

Welp, E, Partanen, T, Kogevinas, M *et al* (1996b)
Exposure to styrene and mortality from non-malignant diseases of the genitourinary system.
Scand. J. Work Environ. Health. **22**, 223-226.

Welp E, Kogevinas M, Andersen A, et al (1996c)
Exposure to styrene and mortality from nervous system diseases and mental disorders.
Am. J. Epidemiol. **144**, 623-633.

WHO(1983)
Environmental Health Criteria: Styrene, No 26.
IPCS, International Programme on Chemical Safety, WHO, Geneva, Switzerland.

Wieczorek, H (1985)
Evaluation of low exposure to styrene. II. Dermal absorption of styrene vapours in humans under experimental conditions.
Int. Arch. Occup. Environ. Health. **57**, 71-75.

Wigaeus, E *et al* (1983)
Exposure to styrene. Uptake, distribution, metabolism and elimination in man.
Scand.J. Work Environ. Health. **9**, 479-488.

Wigaeus, E, Lof, A and Nordqvisit MB (1984)
Uptake, distribution, metabolism, and elimination of styrene in man. A comparison between single exposure and co-exposure with acetone.
British Journal of Industrial Medicine, **41**, 539-546.

Wilson JT, McNabb JF, Wilson RH and Noonan MJ (1983)
Biotransformation of selected organic pollutants in groundwater.
Devel. Ind. Microbio., **24**, 225-233.

Wilson, RH, Hough, GV and McCormick, WE (1948)
Medical problems encountered in the manufacture of American made rubber.
Ind. Med. **17**, 199-207.

Withey, JR (1978)
The toxicity of styrene monomer and its pharmacokinetics and distribution in the rat.
Scand. J. Work. Environ. Health. **4, suppl 2**, 31-40.

Withey, JR (1976)
Quantitative analysis of styrene monomer in polystyrene and foods including some preliminary studies of the uptake and pharmacokinetics of the monomer in rats.
Environ. Health. Perspect. **17**, 125-33.

Withey, JR and Collins, PG (1977)
Pharmacokinetics and distribution oi styrene monomer in rats after intravenous administration.
J Toxicol. Environ. Health. **3**, 1011-1020.

Withey, JR and Collins, PG (1979)
The distribution and pharmacokinetics of styrene monomer in rats by the pulmonary route.
J. Environ. Pathol. Toxicol. **2**, 1329-1342.

Wolf, MA et al (1956)
Toxological studies of certain alkylated benzenes and benzene.
Arch. Ind. Health. **14**, 387-398.

Wolff, MS, Lorimer, WV and Selikoff, IJ (1978b)
Biological indicators of exposure in styrene polymerisation workers. Styrene in blood and adipose tissue and mandelic and phenylgloxylic acids in urine.
Scan. J. work. Environ. **4(suppl.2)**, 114-118.

Wolff, MS, Daum, SM, Lorimer, WV and Selikoff, IJ (1977)
Styrene and related hydrocarbons in subcutaneous fat from polymerisation workers.
J. Toxicol. Environ. Health, **2**, 997.

Wong, O, Trent, LS and Whorton, MD (1994)
An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry.
Occupational and Environmental Medicine. **51**, 386-396.

Wong, O (1990)
A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry.
British J. Ind. Med. **47**, 753-762.

Wong, O and Trent, LS (1999)
Mortality from non-malignant diseases of the respiratory, genitourinary and nervous systems among workers exposed to styrene in the reinforced plastics and composites industry in the United States.
Scand. J. Work environ. Health, **25(4)**, 317-325.

Wuttke, W (2000)
Investigation on the effects of a single I.V application of styrene on pituitary hormones in male rats.
Unpublished report for SIRC.

Yager, JW, Rappaport, SM and Paradisin, WM (1989)
Sister chromatid exchange induced in peripheral lymphocytes of workers exposed to low concentrations of styrene [Abstract-651].
Environ. and Molecular Mutagenesis, **14(15)**, 224.

Yager, JW, Paradisin, WM and Rappaport, SM (1993)
Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene.
Mutation Research. **319**, 155-165.

Yamamoto T, Teramoto K and Horiguchi S (1997)
Effects of styrene on peripheral nerve conduction velocities in rats.
J Occup Health **39**, 319-324.

Yano, BL *et al* (1992)
Abnormal auditory brainstem responses and cochlear pathology in rats induced by an exaggerated styrene exposure regimen.
Toxicologic Pathology **20**, 1-6.

Yasuhara A and Morita M (1987)
Identification of volatile organic components in mussel.
Chemosphere, **16**, 2559-2565.

Yeowell-O'connell, K, Jin, Z and Rappaport, SM (1996)
Determination of albumin and haemoglobin adducts in workers exposed to styrene and styrene oxide.
Cancer Epidemiology, Biomarkers & Prevention, **5(3)**, 205-215.

Yokoyama, K, Araki, S and Murata, K (1992)
Effects of low level styrene exposure on psychological performance in FRP boat laminating workers.
Neurotoxicology. **13**, 551-556.

Young, JD et al (1979)
Pharmacokinetics of inhaled or intraperitoneally administered styrene in rats.
Dev. Toxicol. Environ. Sci. **4**, 297-310.

Unemura T, Kurahashi N, Kondo T, Katakura Y, Sata F, Kawai T and Kishi R (2005)
Acute effects of styrene inhalation on the neuroendocrinological system of rats and the different effects in male and female rats.
Arch Toxicol. **79**, 653-659.

Uuasa, J, Kishi, R, Eguchi, T et al (1996)
Study of urinary mandelic acid concentration and peripheral nerve conduction among styrene workers.
American Journal of Industrial Medicine **30**, 41-47.

Zaidi, NT, Agrawal, AK, Srivastava, SP and Seth, PK (1985)
Effect of gestational and neonatal styrene exposure on dopamine receptors.
Neurobehavioural Toxicology and Teratology, **7**, 23-28.

Zaprianov Z and Bainova A (1979)
Changes in monoamine oxidase activity (MAO) after styrene and ethanol combined treatment of rats.
Activ nerv supp **21**, 262-264.

Zeilhuis, RL (1961)
Systemic toxicity from exposure to epoxy resins, hardeners and styrene.
J Occup. Med. **3**, 25-29.

Zielhuis, RL et al (1963)
The health of workers processing reinforced polyester.
Proc. 14th Int Congress Occup Health, Madrid. 3, 1092-1097.

Zlobina, NS, Izyumova, AS and Ragule, NYu(1975)
The effect of low styrene concentrations on the specific functions of the female organism.
Gig. Tr. Prof. Zabol. **12**, 21-25

Znojemska, S and Svandova, E (1991)
Styrene – Is it a carcinogen?
Krajska Hygienicka Stanice Sterdcceskeho Kraje **43(1)**, 9-12

ANNEX A

IPCS Conceptual Framework for Evaluating a Mode of Action For Styrene Induced Mouse Lung Tumour

Introduction

Styrene inhalation, at exposures ranging from 20 ppm up to 160 ppm, for up to 24 months induced lung tumours in mice (Cruzan *et al*, 2001). Statistically increased incidences of bronchioloalveolar adenomas, but not of carcinomas, were seen in male mice exposed to 40, 80 or 160 ppm styrene for 24 months. No statistically significant increases in lung adenomas were seen in the 20 ppm treatment group and there was no statistically significant dose response relationship. In females, (exposure 22.5 months), the incidence of bronchioloalveolar adenomas was increased significantly in the 20, 40 and 160 ppm treatment groups but with no significant increase at 80 ppm. Only females in the 160 ppm treatment group had a statistically significant increase in bronchioloalveolar carcinomas. No other tissues were affected. In rats however no tumours were observed with inhalation concentrations up to 1000 ppm (Cruzan *et al*, 1998).

In addition to the Cruzan *et al* (2001) study, older investigations in the mouse, but not meeting contemporary standards, have given some indication for possible lung tumour formation after oral application. A critical examination of results from these 4 mouse oral studies has been provided by McConnell and Swenberg (1993), who concluded because of methodological deficiencies and equivocal results the overall data were inadequate to reach any firm conclusions.

Results of eight long term rat studies, in which styrene was given by various dose routes, revealed no tumorigenic effects including no increase in lung tumors. This included an inhalation study, using contemporary protocol design, in which rats were exposed to levels up to 1000 ppm styrene for 24 months (Cruzan *et al*., 1998).

In addition to animal investigations there have been a variety of human epidemiological studies examining cancer incidence in the reinforced plastics industry, the styrene manufacturing industry and the styrene-butadiene rubber industry. These studies have been the subject of several reviews. For example Coggon (1994) concluded that despite the large size of published data it was not possible to rule out a hazard from long term exposures to high exposures (> 50 ppm) styrene. However the data indicated any risk from lower exposure levels was extremely small. In 2002 the Harvard Centre for Risk Analysis also conducted a comprehensive review of potential health risks associated with exposure to styrene (Cohen *et al*, 2002). The science panel put together by the Harvard Center concluded the human epidemiological data did not support a hazard for lung cancer in humans exposed to styrene. The panel also calculated lifetime risk in highly exposed workers and concluded, based on the statistical power of the studies, the balance of epidemiological evidence does not suggest a causal relationship between styrene and any form of cancer. Similarly, after reviewing the available cohort and case-control studies of workers in the GRP industry, the styrene production and styrene-butadiene rubber industry the draft Risk Assessment Report of the UK (United Kingdom, 2002) concludes that these mortality studies provide no evidence for a causative association between styrene exposure and cancer in humans.

The Mode of Action analysis given below relates to mouse lung tumours, the only tumour type clearly induced by styrene in carcinogenicity studies in rats and mice. To assess the significance of the mouse lung tumour data for human health a variety of mechanistic

investigations have been carried out over the past 4 years. These investigations have been directed towards an understanding of the mode of action and aetiology of the mouse lung tumours. The data available up to 2002 have been reviewed by Cruzan *et al* (2000) and an explanation provided for the high sensitivity of mice for lung tumor development as compared to rats and to humans. In view of the complexity of the data from these investigations and the requirement to provide transparency and structure to analysing the mode of action the approach defined in the IPCS framework for evaluating mode of action in chemical carcinogenesis has been used (Sonich-Mullin *et al*, 2001).

Postulated Mode of Action

In the following proposed mode of action data for mice, (species responding to styrene exposure with lung tumours), are compared to those for rats, (species without a carcinogenic effect). This interspecies comparison provides a valuable countercheck whether the proposed mode of action leading to lung tumour formation in the mouse actually differentiates between responding and non-responding species. In the concluding section an extrapolation from mice and rats to humans is presented.

The postulated mode of action is explained by the special cellular and biochemical characteristics of mouse lung which results in the mouse Clara cells producing cytotoxic metabolites of styrene including styrene oxide (SO) and oxidative metabolites of 4-vinyl phenol (4-VP). These metabolites cause early Clara cell toxicity and bronchiolar cell proliferation followed by progressive bronchiolar epithelial hyperplasia and focal crowding of cells. These cellular changes will finally lead to tumour formation.

There are several predisposing factors explaining the species differences in susceptibility to lung tumour formation. These include:

- physiological differences with the mouse having much higher numbers of Clara cells as compared to rats and especially to humans.
- pharmacokinetic differences at the cellular level with mouse Clara cells being more efficient than the lung of rats and humans in oxidative toxification of styrene to SO (including specifically the highly pneumotoxic R-enantiomer) and toxic metabolites of 4-VP.
- detoxification of SO which takes place in rodents both via the microsomal epoxide hydrolase and the cytosolic glutathione S transferase. In humans the latter accounts for only 0.1% while detoxification of SO (if formed at all) is nearly exclusively mediated by epoxide hydrolase. Species differences in these detoxification pathways probably contribute to the higher sensitivity of mice in comparison to rats and humans
- pharmacodynamic differences such as glutathione depletion, (i.e. glutathione depletion is more prominent in mice than in rats), also probably play a role at the cellular level making mouse Clara cells more susceptible to damage. Glutathione depletion is more prominent in mice than in rats and is not expected to occur in humans.

The course of pulmonary effects observed in the chronic mouse styrene inhalation study, i.e. Clara cell toxicity caused by production of styrene metabolites *via* the CYP 2F2 metabolic pathway, cell proliferation, cell tolerance accompanied by changes in Clara cell morphology and biochemistry, (e.g. decreased eosinophilia, loss of apical cytoplasm, focal crowding and reduced CC10 protein), followed sequentially by progressive Clara Cell hyperplasia is consistent with a non-genotoxic multistage mode of action.

When looking at these relative susceptibilities it is clear that the mouse carries all of the factors for tumour production whereas humans and rats do not share these predisposing factors.

Key Events

The key events considered with respect to styrene lung tumourgenesis in the mouse include:

- *Clara cells are the target cells for lung toxicity and proliferation*. Species susceptibility correlates with the number of Clara cells. For example an examination of distribution and numbers of Clara cells showed that in the mouse these cells are distributed from the terminal bronchioles to the trachea while in rat they are found only in the terminal and distal bronchioles. In the mouse Clara cells comprise about 85% of bronchiolar epithelium whereas in the rat only about 25% of the bronchiolar epithelium are Clara cells (Plopper *et al*, 1980).

- *Formation of the toxic metabolites in Clara cells is mediated via Cytochrome CYP 2F2 a pathway more pronounced in mice than in rats*. A variety of investigations have demonstrated that Clara cell toxicity in the mouse is mediated by CYP 2F2 generated metabolites such as SO (including specifically the highly pneumotoxic R-enantiomer) and oxidative metabolites of 4-vinyl phenol (4-VP) (Cruzan *et al,* 2002, 2004). With regard to the formation of the different SO enantiomers Gadberry *et al* (1996) demonstrated that the R-enantiomer is a more potent pneumotoxicant and hepatotoxicant than the S-enantiomer. Various investigations have been undertaken examining the formation of the different isomers in different species and tissues. For example, Hynes *et al* (1999) demonstrated that mouse Clara cells produce about 3-times more of R-SO than of S-SO, while the rat produces more of the S-enantiomer. Overall, mouse Clara cells produce 15-times more of R-SO than rat Clara cells. In addition to species differences in pulmonary SO formation there is also a clear inter-species difference in the formation downstream metabolites of 4-VP, which are highly toxic to mouse Clara cells (see below). Studies in which styrene was incubated with lung microsomes of mice and rats in the presence of glutathione showed a clear species difference in the formation of the toxic 4-VP ”downstream” metabolites in the order of mice > rats (Bartels, 2004). This further “downstream” metabolism of 4-VP will occur either from further side chain oxidation and, perhaps more importantly, from further aromatic hydroxylation to a catechol derivative which can easily undergo autoxidation to highly reactive o-Quinones.

- *Cytochrome CYP 2F2 mediated metabolites of styrene, i.e. styrene oxide and the downstream metabolites of 4-VP, are highly toxic to mouse Clara cells but the effect on rat lung is minimal*. To investigate whether the cell damage and proliferation was associated with formation of oxidative metabolites rather than parent styrene Green *et al*, (2001) treated mice with 5-Phenyl-1-pentyne, a cytochrome P450 inhibitor prior to exposing to styrene (40 or 160 ppm, 6 hours per day for 3 days). The results of the study showed without the inhibitor cellular damage and increases in cell division in the lung of mice. In rats such effects were not observed at exposure levels up to 500 ppm. Pretreatment with the inhibitor protected against the toxic effects in mice indicating that the pulmonary pathological changes are caused by a toxic metabolite(s) formed by the cytochrome P-450 metabolism of styrene. The role of individual styrene metabolites in producing Clara cell toxicity has been examined by Kaufmann *et al* (2004) after i.p. application over 3 days. In summary, treatment of mice with styrene oxide and 4-VP caused up to 19-fold increases in cell proliferation in the large, medium and terminal bronchioles. Cell proliferation at the high dose was associated with toxic injury to Clara cells and regeneration, while at a lower dose level only cell proliferation without toxicity

was observed. Treatment also caused glutathione depletion with an up to a 50% reduction in the number of Clara cells staining for glutathione (see below). Histopathological changes were characteristic of those seen following styrene exposure, i.e. flattened cells with loss of apical buldging into the bronchial lumena (Kaufmann *et al*, 2004). Other experiments with 4-VP substantiated the pronounced effects of this metabolite on bronchiolar cell proliferation. For example, Cruzan *et al*, (2004) reported hyperplasia of the terminal bronchioles in mice treated intraperitoneally at 6, 20 and 60 mg/kg/day for 14 consecutive days. The NOAEL was 2 mg/kg/day. No effects were however seen in the lungs of rats treated at 60 mg/kg/day or in the liver of mice or rats. A single high i.p. dose of 4-VP (100 or 150 mg /kg) in mice produced prolonged lung damage as demonstrated by effects on the broncho-alveolar lavage fluid (BALF) (Carlson *et al* 2001, 2002). At the single high i.p. dose of 150 mg/kg lung toxicity was found by BALF in rats, too (Carlson *et al*, 2001). Pre-treatment of mice with 5-phenyl-1-pentyne, (inhibitor of CYP 2F2), or with Diethyldithiocarbamate prevented the toxicity indicating that a further metabolism is required to produce the toxic species (Carlson, 2002). Diethyldithiocarbamate was primarily used as an inhibitor of CYP 2E1 (Carlson, 2002) but subsequently it was shown that it also acts on other cytochromes (Carlson 2003, 2004). Studies with other styrene metabolites specifically following the ß-oxidation pathway failed to cause Clara cell toxicity (see Other Modes of Action).

- *Differences in the detoxification pathways may contribute to the higher sensitivity of mice.* The detoxifiation pathways of SO via epoxide hydrolase and glutathione S-transferase are qualitatively similar in mice and rats. A detailed analysis of the metabolic constants shows that epoxide hydrolase mediated detoxification is less effective in the lungs of mice as compared to rats. On the other hand, glutathione S-transferase activity is higher in mice than in rats (Filser *et al*. 1999).

- *Glutathione depletion in the lung as a contributing factor to cytoxicity and lung tumour development is more pronounced in mice than in.* Studies of glutathione conjugation in the mouse lung have shown that mouse pulmonary tissue is susceptible to styrene induced glutathione depletion. Filser *et al* (1999, 2002) using measured data and PBPK modelling reported lung glutathione levels of mice exposed to styrene, (300ppm), for 6 hours/day for up to 3 days. After a single 6-hour exposure glutathione levels were reduced by 20%, which by day 3 had declined to about 60% of that seen in untreated mice. Similar treatments produced only minor effects on glutathione levels in the rat. Furthermore, Kaufmann *et al* (2004) showed histopathologically by a specific glutathione stain that SO and 4-VP caused glutathione depletion in the bronchiolar epithelium of mice after intraperitoneal application over 3 days. Overall, it is highly likely that the combination of both SO and 4-VP conjugation results in glutathione depletion at the cellular level in the mouse. Based on experience with other mouse Clara cell toxicants such as Coumarin (Vasallo *et al*, 2000) it is likely that glutathione depletion plays an important contributing role in lung tumour formation in the mouse. The biological relevance of glutathione depletion is decreasing from mice to rats.

- *Tumour formation is a late event (i.e. not observed at 12 or 18 months) associated with progressive hyperplasia in terminal bronchioles*. As has been discussed earlier Clara cell toxicity, followed by extensive cell replication and subsequent hyperplasia with focal crowding appear to play key roles in development of lung tumours in the mouse. No lung pathology has been seen in the rat. While Clara cell toxicity and cell replication moderated with continued styrene exposure (probably as the Clara cells became more tolerant to styrene), clear evidence of hyperplasia was seen in mice following 12 and 18 months of exposure (Cruzan *et al*, 2001). As no changes were ever observed in the

alveoli the Clara cell pathology is both a species (mouse) and cell (Clara cell) specific phenomenon.

Dose-Response Relationship

The chronic inhalation study in the mouse while indicating that exposure of mice to styrene via inhalation increases the incidence of lung tumours did not show a statistically significant dose-response relationship. i.e. % female and male mice with lung tumours being 12, 32, 34, 22 and 54% and 34, 48, 72, 60 and 72% respectively at doses 0, 20, 40, 80 and 160 ppm (Cruzan *et al*, 2001). This lack of a clear dose-response relationship is reflected in the Key Events leading to tumour formation.

- *Clara cells are the target cells for lung toxicity and cell proliferation.* The lack of a clear dose response relationship in tumour development is mirrored in the lack of a clear dose response for the effects on Clara cells in the mouse. For example, Green *et al* (2001) exposed mice for up to 10 days with styrene at 0, 40 or 160 ppm. At various points pulmonary labelling indices (using BrdU labelling technique) were measured in large and terminal bronchioles of exposed mice. Although treatment at 160 ppm produced a slightly larger labelling index than was seen at 40 ppm both treatments produced significant increases as compared to controls. Similarly, Cruzan *et al* (1997) in a study in which mice were exposed to styrene for 2, 5 or 13 weeks at 0, 50, 100, 150 or 200 ppm found histopathological changes, (i.e. decreased eosinophilia and focal crowding) in bronchial epithelium in the majority of mice in all treatment groups. Studies examining labelling indices found higher labelling index at 100 or 200 ppm as compared with lower doses. Similar to the tumour incidence there appears to be only a weak dose response relationship. Differences and variability in individual susceptibility to styrene in mice explains the lack of a clear dose response relationship to Clara cell toxicity and resulting tumour formation. No lung effects were seen in rats up to 1500 ppm after 13 weeks.

- *Formation of the toxic metabolites in Clara cells is mediated via Cytochrome CYP 2F2.* As described above CYP 2F2 present in the mouse Clara cells is responsible for metabolising styrene to cytotoxic metabolites. Pre-dosing mice with the inhibitor of CYP 2F2, 5-phenyl-1-pentyne, is effective at preventing Clara cell toxicity and proliferation. There is evidence that the levels of the mouse Clara cell toxicant, SO, increases proportionally in mouse lung tissue with increasing styrene exposure. In a species comparison with ventilated perfused lungs of mice and rats Filser (2004) concluded that at styrene exposures up to 500 ppm the levels of SO in mouse pulmonary tissues are at least 3-fold higher than those in rat lung. However the lung burden of SO in mice at styrene exposures producing lung tumours, e.g. 160 ppm are similar to SO lung burden in rats at 1000 ppm styrene exposure which was without effect. While such data support the fact that tumour induction in the mouse is not a genotoxic phenomenon it raises question about why mouse Clara cells are so sensitive to oxidative metabolites of styrene including SO. A possible reason again relates to the fact that the measures of SO formation are an aggregate assessment based on the total lung burden whereas in fact the interest is at the Clara cell level. The importance of considering events at the cellular level rather than the tissue level has been highlighted by Green (2000) who exposed mice to 0, 40 or 160 ppm styrene for up to 6 hours after which lungs were excised and one lobe used to estimate CYP 2F2 levels with Western blotting while another lobe was subjected histopathological examination. Pulmonary CYP 2F2 was found to be remarkably variable with up to 5-fold differences between animals. There

was also no correlation between histopathological changes and levels of CYP 2F2 in individual animals. Thus while the presence of CYP 2F2 is necessary to generate toxic metabolites there is no simple relationship between metabolism and morphological damage which explains why some mice in the 40 ppm treatment group showed more severe histopathological effects than did mice in the 160 ppm treatment group. An explanation for the variability in histopathology probably lies not only in the presence of the enzymes for styrene activation but also in the ability of the cell to detoxify the toxic metabolites. This complexity of toxification and detoxification in the mouse Clara cell again explains the lack of a clear dose-response relationship in tumour development.

- *Glutathione depletion in the lungs is a contributing factor to cytotoxicity and lung tumour formation*. Filser *et al* (1999) reported both measured and modelled glutathione concentrations in the lungs of mice and rats exposed to 300 ppm styrene for 6 hours. After a single exposure glutathione levels in mouse was reduced by 20% and after 3 days exposure reductions had increased to 60 %. At 40 ppm there was however no statistical differences between glutathione levels in the lungs of styrene treated and control mice. At 80 ppm styrene treatment there was a statistically significant decrease in glutathione levels on the second day of exposure. At 160 ppm greater reductions in mouse pulmonary glutathione levels were reported. In rats glutathione reductions in lungs only became statistically significant at exposure concentrations of 300 ppm and above. The lack of aggregate glutathione depletion in pulmonary tissue at styrene exposures associated with pulmonary toxicity has raised a question about the biological relevance of glutathione depletion in mouse lung toxicity and development of pulmonary tumours. It is important to remember that all measurements were of aggregate glutathione levels in the lung as a total. Examination of lung from mice exposed to styrene have however shown large variations in glutathione levels at the cellular level, (Kaufmann *et al* 2004) with, in the same animal, some Clara cells being devoid of glutathione while other cells showed normal levels. Because of variations in individual animals and individual cells within the same animals it is difficult to show a clear dose response effect for glutathione depletion especially at lower dose levels. Again the lack of a clear dose-response in glutathione depletion is consistent with the lack of a clear dose response in tumour formation.

- *Tumour formation is a late event, (i.e. not observed at 12 or 18 months), associated with progressive hyperplasia in terminal bronchioles.* The chronic inhalation study in the mouse reported by Cruzan *et al* (2001showed a time related and dose-related increase in bronchiolar hyperplasia in the terminal bronchioles and bronchiolar epithelial hyperplasia extending into the alveolar ducts. Results of interim sacrifices indicated the presence of these hyperplasias in mice exposed to 160 ppm styrene for 12 months and in mice exposed to 40 ppm styrene for 18 months. These hyperplastic changes finally transformed into lung tumors at the end of the exposure period. Again these data show no clear dose-response but the data do indicate a concentration*time dependence.

Temporal Associations

To develop an understanding of the temporal changes in lung pathology following long term inhalation exposure to styrene groups of mice were killed 2, 5, 13, 52 and 78 weeks after start of treatment. Histopathological examination of the lungs showed a steady progression of the lesions, starting with decreased eosinophilia and focal crowding of nonciliated cells in the bronchiolar epithelium continuing to bronchiolar hyperplasia in the terminal bronchioles extending finally into the alveolar ducts. At the end of the 2 years of treatment all mice at all

doses levels were affected. Only at the study termination increases in the incidence of lung tumors were observed (Cruzan *et al*, 2001).

Various investigations have characterized the time course of cellular changes in mouse lung following repeated inhalation exposures to styrene. For example Cruzan *et al* (1997) conducted a study in which in which CD-1 mice were exposed to 0, 50, 100, 150 and 200 ppm styrene for up to 13 weeks. Some mice were also killed after 2 and 5 weeks of exposure. Histopathological changes to the bronchiolar epithelium were characterized by decreased eosinophilia and focal crowding of Clara cells in the bronchiolar epithelium considered to represent cell proliferation. A study of cell proliferation using BrdU labelling identified a statistically significant increase in Clara cell replication (doses 150 and 200 ppm) after 2 weeks of treatments with an approximate 4-fold increase in percentage of labelled cells as compared to untreated controls. After 5 weeks of treatment a non significant increase (isolated animals) was seen in the treated mice while at 13 weeks no increase in the percentage of labelled cells was seen. The large variations in the labelling index among animals seen in the investigation are consistent with the poor dose response relationship for tumour formation as mentioned above. The cell proliferation was only seen in Clara cells with no effects in toxicity or increased BrdU labelling being seen in the Type II pneumocytes (Cruzan *et al*, 1997). These findings are similar to those reported by Roycroft *et al* (1992) who studied pulmonary changes in $B_6C_3F_1$ mice exposed to up to 500 ppm styrene.

Biological Plausibility & Coherance

The postulated mode of action for the mouse lung tumours induced by inhalation exposure to styrene is consistent with what is known about the metabolic competence of mouse Clara cells and their capacity to form reactive intermediates by the CYP 2F2 metabolic pathway (Cruzan et al, 2002). It is widely accepted that toxicity and mitogenesis are of critical importance in expression of non-genotoxic carcinogenicity and sustained regenerating cell division secondary to cytotoxicity is accepted as a mode of action for many non-genotoxic carcinogens. The time course of pulmonary effects observed in the chronic mouse styrene inhalation study, i.e. Clara cell toxicity, cell proliferation, changes in Clara cell morphology, (e.g. decreased eosinophilia, loss of apical cytoplasm and focal crowding), followed sequentially by Clara Cell hyperplasia is consistent with a non-genotoxic multistage mode of action.

The data show that mouse Clara cells develop a tolerance to cytotoxicity and cell replication with repeated styrene exposure. This tolerance is not only associated with a change in Clara cell morphology as described above but also with a change in the biochemistry of the cells. For example Gamer *et al* (2000) reported changes in both glutathione and CC10 protein levels in mice treated with styrene for up to 28 days. Similar results have been seen with the mouse Clara cell toxicant coumarin which has also been reported to produce reductions in glutathione levels, loss of cell secretory protein CC10 (CC16) and increases in GGT, (gamma glutamyl transpeptidase), activity (Vassallo *et al*, 2000). It has been proposed that the development of Clara cell tolerance to cytotoxic chemicals such as coumarin is accompanied by both morphological and biochemical changes in the cell and may be an important step in the multi-step progression to tumour development.

Other Modes of Action

Genotoxicity as a Mode of Action

A series of experiments have been directed to the question of whether or not genotoxicity may play a role in tumour development. Specifically investigations were carried out to determine if:

- any adduct formation is high enough to explain tumour formation
- there is a difference in adduct formation between rats and mice or
- there is a difference adduct formation between target (lung) and non-target tissues (liver).

A post-labelling experiment with an inhalation exposure of two weeks did not lead to DNA-binding (detection limit 3-5 adducts / 10^7) in mice at 40 and 160 ppm and in rats at 500 ppm (Ottender *et al*, 1999) while postlabelling studies in the livers and lungs of mice and rats failed to find any correlation between tumour development and DNA adduct formation (Ottender *et al*, 2002). In a second experiment with higher sensitivity rats and mice were exposed to ^{14}C-Styrene, (with the highest possible specific radioactivity). Exposure was over a 6- hour period at 160 ppm (Boogaard *et al*, 2000). In this latter investigation minimal DNA-binding (1-5 adducts /10^8) was detected. This low level of adduct formation could not account for lung tumour formation.

In addition there was no difference in DNA-binding of rats and mice (i.e. no species specificity) and DNA binding was less in the lung as compared to the liver (i.e. no tissue specificity).

Such data indicate that genotoxicity is unlikely to be involved in lung tumour formation.

Pneumotoxicity of Metabolites from ß–Oxidation Metabolic Pathway as a Mode of Action

An examination of on urinary metabolites (Johanson *et al*, 2000 and *Sumner et al*, 2001) showed that the ß -oxidation pathway (leading to 2-phenylethanol and via the aldehyde to phenylacetic acid) is a more prominent metabolic pathway in mice as compared to rats. Studies were therefore undertaken to explore the possibility that metabolites from the ß-oxidation pathway could be pneumotoxic to Clara cells thus helping to explain species differences. A series of investigations in mice were carried out by carried out by Kaufmann *et al* (2004) to measure lung cell proliferation after intraperitoneal application of the metabolites from the ß-oxidation pathway 2-phenylethanol, phenylacetaldehyde and phenylacetic acid. In addition, 1-phenylethanol and acetophenone were given. None of the metabolites produced Clara cell damage or cellular proliferation in the bronchiolar epithelium. Although treatment with the metabolites did produce an increased proliferation of the alveolar cells the response is not considered relevant to tumour induction as no evidence of styrene related pathological effects have been seen the alveoli of mice (or rats) at any exposure concentration or at any time point. Thus, this pathway cannot account for lung tumour formation starting from the bronchiolar epithelium.

Oxidative Stress as a Mode of Action

To examine the possibility that oxidative stress may be playing a role in tumour development female mice were exposed to styrene at concentrations of 40 or 160 ppm (Gamer *et al*, 2000). Animals were killed after 1, 5 or 20 exposures and the lungs examined by light and electron microscopy for histopathological effects and lung lavage fluid was examined for cell counts, protein, lactate dehydrogenase, alkaline phosphatase, N-acetyl-ß-D-glucuronidase, glutamyl transferase, glutathione, CC 16 protein (marker for Clara cells) and lysozyme. Lung homogenates were analysed for 8-OH-deoxyguanosine in lung DNA, malondialdehyde, catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase and glutathione as markers of oxidative stress. Finally CC16 protein was measured in blood serum.

Although a depletion of glutathione was seen in lung homogenates after 20 exposures there was no evidence of substantial oxidative stress as indicated by unchanged levels of 8-OH-deoxyguanosine, superoxide dismutase, malondialdehyde, catalase, glutathione reductase,

and glutathione peroxidase. Examination of lavage fluid provided no indication of inflammatory responses. A concentration-time related increase in activity of glutamyltransferase indicating cytotoxicity to Clara cells was present over the study period with a maximum level after 5 exposures. Clara cell involvement was substantiated by reductions in concentrations of CC16 protein in the lavage fluid and in blood serum throughout the study.

Histopathological changes in the lung after a single treatment were characterized by vacuolation and desquamation of secretory cells in the large and medium bronchioles. After 5 and 20 exposures cellular crowding, indicative of early hyperplasia was seen in the large and medium bronchioles. Electron microscopy showed Clara cells were the main target cells. After 20 days of damage phenotypic changes were noted in the Clara cell population with some of the cells having normal features of Clara cells but with others exhibiting slightly different features such as absence of apical blebs and reduction in secretory granules. Overall, the data do not support the hypothesis that substantial or sustained oxidative stress plays a role in styrene induced tumour development.

In summary three other potential modes of action were investigated. The results of these investigations show genotoxicity is not a key factor in tumour development. Styrene metabolites generated via the ß-oxidation pathway also play no role in Clara cell toxicity and oxidative stress can be excluded as a mode of action.

Assessment of Postulated Mode of Action

Overall the results of investigative studies on styrene indicate that the susceptibility of mice to development of lung tumours is linked to the following factors:

- A greater number of Clara cells in mouse pulmonary tissues as compared to rats.
- Mouse Clara cells contain high levels of the cytochrome P-450 isoform, CYP 2F2 responsible for metabolising styrene to metabolites highly toxic to mouse Clara cells, (i.e. styrene oxide and oxidative metabolites of 4-VP). This metabolic toxification occurs much less in rats. These metabolites have been shown to lead to early lung toxicity and cell proliferation in mice, but such effects have not been found in rats.
- The detoxification pathways involved may not be efficient enough in mice and thereby can lead at the cellular level to relatively high levels of the toxic species described above as compared to the rat.
- Pharmacodynamic differences at the cellular level, probably related to glutathione depletion, may account for the susceptibility of mouse Clara cells to cellular injury whereas rats are more resistant.

These factors are consistent with styrene selectively targeting mouse Clara cells causing cytotoxicity and increased cell replication rates. While these early effects moderate with continued exposure, (presumably as Clara cells develop some tolerance to styrene toxicity), clear evidence of bronchiolar epithelial hyperplasia is seen in mouse lung following 12 and 18 months of exposure (Cruzan *et al*, 2001).

The lack of histpathological effects in the alveoli of the mouse is consistent with the fact that Type II pneumocytes do not possess CYP 2F2 required to generate the toxic metabolites of styrene.

The morphological effects, (including the change in phenotype and the absence of tumours at 18 months indicate a late developing tumour consistent with progressive hyperplasia), can be linked directly to the CYP 2F2 mediated metabolism of styrene producing metabolites

toxic to mouse Clara cells. Thus both the histomorphological and biochemical data provide a high level of support for the postulated mode of action.

Uncertainties, Inconsistencies and Data Gaps

Measured kinetic data has enabled the development of PBPK-models based on SO formation (Sarangapani *et al*, 2002 and Csanardy *et al*, 2003). The model of Sarangapani *et al* (2002) specifically estimated styrene and SO levels in the terminal bronchioles vs. whole lung. It predicts a 2-fold higher level of the R-SO isomer in mouse lung at 40 ppm than in rat lung at 1000 ppm. But it seems improbable whether this difference in one specific isomer solely would explain the marked species difference.

Overall, while the models have slight differences they have led to qualitatively similar results:

- The exposure of lung cells to SO predominantly results from pulmonary metabolism of styrene with minimal contribution from SO formed in the liver.
- At relatively low styrene inhalation exposures the local concentration of SO (including the R-isomer) in the lung is higher in mice than in rats.

Overall SO lung burden (specifically R-SO lung burden) is higher in mice than in rats.

While the mouse may have greater metabolic capacity for conversion of styrene to styrene oxide and its R-isomer the pharmacokinetic data shows that the pulmonary concentrations of SO in mouse exposed to 40 ppm, (exposure producing lung tumours), is not much different from pulmonary concentrations of SO in rats exposed to 1000 ppm where no excess of lung tumours were seen. Thus it has been proposed that the aggregate measures of SO exposure in the lung do not appear to sufficiently explain the relative susceptibilities of the mouse and rat for lung tumour development (Cohen *et al*, 2002 and Filser, 2004).

The proposal that glutathione depletion may play an important role in mouse Clara cell toxicity and subsequent carcinogenesis has also been questioned. For example Cohen *et al* (2002) pointed out that exposures to 40 ppm of styrene did not produce significant glutathione depletion in mouse lung but such treatment did produce hyperplasia and even tumours. As described earlier it is however important to differentiate effects at the tissue level and effects at the cellular level. At the tissue level lung surfactant contains quite high levels of glutathione especially for “scavenging” reactive species while at the cellular levels glutathione can vary 4-fold between individual mouse Clara cells. This heterogeneity helps explain why in studies with 4-VP approximately 50% of mouse Clara cells did not stain for glutathione while the other 50% stained with a similar intensity to untreated controls. Thus at the tissue level there may be no apparent change in glutathione whereas at the cellular level individual cells may have low if any ability to conjugate reactive species making them extremely susceptible to damage.

To date the formation and metabolism of 4-VP, including the striking semi-quantitative species differences, has only been investigated *in vitro.* How this may relate in quantitative terms to the *in vivo* situation and especially to the levels in the target cells, the Clara cells, requires study. Taking account of the metabolic instability of the 4-VP downstream metabolites and the predominant importance of their concentrations at the cellular levels as compared to the “aggregate” total lung or even the whole body, it is not expected that such studies will be accomplished easily. Nevertheless, such studies are of importance in refining the mode of action.

Even in the absence of data from such studies there is compelling evidence that mouse Clara cells possess unique properties, which come together or act in combination for lung tumour development in mice. These unique properties include:

1. sensitivity to the cytotoxic effects of SO, including specifically R-SO,

2. sensitivity to cytotoxic effects of 4-VP metabolites,

3. glutathione depletion in individual Clara cells

4. cellular regeneration and development of tolerance resulting in morphological and biochemical changes to the Clara cell, and

5. long term hyperplasia with cellular/focal crowding.

None of these properties are operational in the rat and hence the rat does not develop lung tumours.

Conclusion and extrapolation to humans

Sensitive *in vivo* DNA-binding studies in mice and rats have shown that genotoxicity is most unlikely to be the driving force for lung tumour development in mice. If a non-genotoxic mechanism is operative, a threshold (or a highly non-linear) dose response relationship can be assumed and three questions required answers:

1. what mechanism is leading to lung tumors in mice?
2. why do lung tumors do not develop in rats?
3. what sensitivity is to be assumed for humans in relation to mice and rats?

Answers to questions 1. and 2. have been provided in the preceeding sections. Basically the high sensitivity of mice in comparison to the non-responsiveness of rats for lung tumour induction depends on multiple factors:

1) Clara cells in mice both generate the toxic metabolites as well as being the target cells for these metabolites. The number of Clara cells is much higher in mice than rats.

2) There are two oxidative pathways for formation of toxic metabolites:

a) formation of SO is more pronounced in the lung of mice while the detoxification of SO may be more effective in rats. In total the local concentration of SO is higher in mice than in rats

b) 4-VP and its downstream metabolites, which are cytotoxic for mouse Clara cells, are formed to a greater extent in the lungs of mice as compared to the rat.

3) These metabolites lead to greater glutathione depletion in mice than in rats: glutathione depletion may be a contributing factor for tumour formation.

4) These factors lead to an increase in cell proliferation and histopathological changes as pre-stages of lung tumour development in the mouse. Inhibition of oxidative metabolism in mice prevents Clara cell cytotoxicity and proliferation in this species as the first steps in tumour formation.

The lack of tumour formation in the rat is explained by the fact that rats do not produce the necessary cellular concentrations of toxic metabolites to cause Clara cell toxicity and replication resulting in the morphological and biochemical changes in Clara cells as a first stage in the multi-stage process to tumour development. The resistance of the rat and the sensitivity of the mouse to Clara cell toxicity are explained, as described above, by the physiological, pharmacokinetic and pharmacodynamic differences between the species.

The answer to the question about human sensitivity is found in the fact that the Key Events for the postulated mode of action are even less operative in humans as compared with the non-responsive rat. For example:

1. The number of Clara cells, (being both responsible for the formation of toxic metabolites and the target fore their toxic action), is very low in humans, even less than in rats. While Clara cells comprise about 85% of bronchiolar epithelium in mice and 25% in rats, in humans such cells are rare (Plopper *et al*, 1980).
2. The level of the enzyme CYP 2F2 required for the formation of the Clara cell toxicants such as SO, (including the highly pneumotoxic R-enantiomer), and the downstream metabolites of 4-VP occurs at best only to a negligible extent in humans. Studies with human lung tissues have shown a general lack of CYP 2F2 activity in human pulmonary tissue leading to the formation of SO. In a study of 38 human lung samples, Nakajima *et al* (1994) found only low levels of cytochrome P450 dependent monoxygenase activity in some of the samples; the highest level measured was however approximately 400-fold lower than the average measurement in rat lung. Carlson *et al* (2000) reported only a slight styrene metabolising capability in 1 of 8 human lung samples while Filser *et al* (2002) failed to find any evidence of CYP 2F2 activity in human lung samples and formation of SO.
3. In human lung, detoxification of SO (if formed at all in human pulmonary tissue) takes place predominantly *via* epoxide hydrolase (located on the endoplasmatic reticulum in close proximity to the toxifying cytochrome P450s). This close proximity of the “detoxifying” enzymes to any “toxifying” enzymes ensures the efficient removal of any toxic metabolites. Rodents use both epoxide hydrolase and glutathione-S-transferase as detoxification pathways with the mouse relying on glutathione conjugation more so than the rat. As glutathione-S-transferase is located in the cytosol this makes this detoxification pathway less efficient than the epoxide hydrolase pathway. In comparison to the rodent species, humans represent an extreme as SO detoxification nearly exclusively proceeds via epoxide hydrolase and glutathione S-transferase accounts for only approx. 0.1% of SO detoxification.
4. Taking account both of the toxification to SO and its detoxification, PBPK-modelling by two approaches (Sarangapani *et al*, 2002 and Csanardy *et al*, 2003) have shown that the SO content of human lungs must be very small, if there is any.
5. Formation of 4-VP and its downstream metabolites in humans is less than in rats and much less than in mice (Bartels, 2004). This has been demonstrated by a comparative incubation of lung microsomes from mice, rats and humans using glutathione as a “trapping” reagent for the reactive, unstable downstream metabolites of 4-VP. Such metabolites are formed either from further side chain oxidation or more importantly from further aromatic hydroxylation to a highly reactive catechol derivative.
6. Glutathione depletion caused by SO does not to occur in humans. This has been demonstrated by PBPK-modelling by Filser *et al* (1999, 2002) using the biokinetic data for the toxification of styrene to SO and the detoxification of the latter *via* epoxide hydrolase and glutathione-S-transferase. As reactive downstream metabolites of 4-VP are formed in human lung only to a very small extent (Bartels

2004), the 4-VP metabolic pathway is not expected to cause any glutathione depletion in human pulmonary tissue.

As all of the Key Events are not, (or at best only to a negligible extent), operative in humans, the early histopathological and biochemical changes leading ultimately to tumours are not to be expected to occur in human lung.

References

Bartels, M., Preliminary report from Dow Chemical Company, 4-VP and Styrene Metabolism Study In Lung and Liver Microsomes (2004).

Boogaard, P. J., Kloe, K. P., Wong, B. A., Sumner, S. C., Watson, W. P., and Sittert, N. J. Quantification of DNA Adducts Formed in Liver, Lungs and Isolated Lung Cells of Rats and Mice Exposed to 14C-Styrene by Nose Only Inhalation. Toxicol. Sci.57: 203-216 (2000).

Carlson, G.P. Effect of the Inhibition of Metabolism of 4-Vinylphenol on its Hepatotoxicity and Pneumotoxicity in Ras and Mice. Toxicology 179, 129-136 (2002).

Carlson, G.P. In Vitro Metabolism of Styrene to Styrene Oxide in Liver and Lung of CYP2E1 Knockout Mice. Toxicol. Environ. Health. Part A, 66: 861-869 (2003).

Carlson, G.P. Influence of Selected Inhibitors on the Metabolism of the Styrene Metabolite 4-Vinylphenol in Wild-Type and CYP2E1 Knockout Mice. Toxicol. Environ. Health. Part A, 67: 905-909 (2004).

Carlson, G. P., Mantick, N.A. and Powley, M. Metabolism of Styrene by Human Liver and Lung. J. Toxicol. Environ. Health. 59: 591 – 595 (2000).

Carlson, G. P., Perez Rivera, A. A., and Mantick, N. A. Metabolism of the Styrene Metabolite 4-Vinylphenol by Rat and Mouse Liver and Lung. J. Toxicol. Environ. Health, Part A, 63, 541-551 (2001).Carlson, G.P., Ullman, M., Mantick, N. A. and Snyder, W. 4-Vinylphenol-Induced Pneumotoxicity and Hepatotoxicity in Mice. Toxicol. Pathol. 30: 565-569 (2002).

Cohen, J. T., Carlson, G., Charnley, G., Coggon, D., Delzell, E., Graham, J.D., Greim, H., Krewsi, D., Medinsky, M., Monson, R., Paustenbach, D., Rappaport, S., Rhomberg, L., Ryan, P.B. and Thompson, K. A Comprehensive Evaluation of the Potential Health Risks Associated with Occupational Exposure and Environmental Exposure to Styrene. J. Toxicol. Environ. Health. 5, 1-267 (2002).

Coggon, C D. Epimiological Studies of Styrene-Exposed Populations. Critical Reviews in Toxicology. 24: 107-116 (1994).

Cruzan, G., Cushman, J. R., Andrews, L. S., Granville, G. C., Miller, R.R., Hardy, C. J., Coombs, D. W. and Mullins, P. A. Subchronic Inhalation studies of Styrene in CD Rats and CD-1 Mice. Fund. Appl. Toxicol. 35, 152-165 (1997).

Cruzan, G, Cushman, J.r., Andrews, L.S., Granville, G. C., Miller, R. R., Hardy, J. C., Coombs, D. W., Mullins, P. A. and Brown, W. R. Chronic Toxicity/Oncogenicity Study of Styrene in CD Rats by Inhalation Exposure for 104 Weeks. Toxicol. Sci. 46, 266-281 (1998).

Cruzan, G., Cushman, J.r., Andrews, L.S., Granville, G. C., Miller, R. R., Hardy, J. C., Coombs, D. W., Mullins, P. A. and Brown, W. R. Chronic Toxicity/Oncogenicity Study of Styrene in CD-1` Mice by Inhalation Exposure for 104 Weeks. J. Appl. Toxicol. 21, 185-198 (2001).

Cruzan,G., Carlson, G.P., Johnson, K.A., Andrews, L.S., Banton, M.I., Bevan, C., Cushman, J.R.Styrene Respiratory Tract Toxicity and Mouse Lung Tumors are Mediated by CYP2F-Generated Metabolites. Reg. Toxicol. Pharmacol. 35, 308-319 (2002).

Cruzan, G., Carlson, G. P., Turner, M. And Mellert, W. Ring-Oxidized Metabolites of Styrene Contribute to Styrene Induced Clara Cell Toxicity in Mice. Accepted for Publication in J. Toxicol. Environ. Health (2004).

Csanardy, G. A., Kessler, W., Hoffmann, H. D. and Filser, J. G.,. A Toxicokinetic Model for Styrene and its Metabolite Styrene 7-8 Oxide in Mouse, Rat and Humans with Special Emphasis on the Lung. Toxicology Letter. 138: 75-102 (2003).

Filser, J. G., Oberste-Frielinghaus, M., Dhawan-Robl. M., Faller, T. H., Kessler, W and Csanady, G. A. A Physiological Toxicokinetic Model for Styrene and Styrene-7,8-Oxide in Mouse, Rat and Human Considering Metabolism in the Lung, Preliminary Report (1999).

Filser, J. G, Kessler, W and Csanady, G. A. Estimation of a possible tumourigenic risk of styrene from daily intake via food and ambient air, Toxicol. Letters 126: 1-18 (2002).

Filser, J.G. Styrene-7,8-oxide burden in ventilated perfused lungs, final draft report (2004).

Gamer, A. O., Leibold, E., Deckardt, K., Kittel, B and Hildebrand, B. Styrene Subacute Vapour Inhalation Study in Female CD-1 Mice. Investigations on Oxidative Stress and Cytototoxicity in the Lung. BASF Project No. 9910284/99039 (2000).

Gadberry, M. G., DeNicola, D.B. and Carlson, G.P. Pneumotoxicity and Hepatotoxicity of Styrene to Styrene Oxide. J. Toxicol. Environ. Health 48: 273-294 (1996).

Green, T. The Role of Cytochrome P450 in Styrene-Induced Nasal Lesions in the Mouse. Report No: CTL/R/1418 (1999)

Green, T. The Effects of Styrene on the Mouse Lung After Exposures of 2, 4 and 6 Hours, Report No: CTL/R/1462 (2000) (ALAN CHECK - WERE DOES THIS BELONG TO?)

Green, T., Toghill, A. and Foster, J. R. The Role of Cytochrome P450 in Styrene Induced Pulmonary Toxicity and Carcinogenicity in the Mouse. Toxicology, 169: 107-117 (2001).

Hynes, D. E., DeNicola, D. B. and Carlson, G. P. Metabolism of Styrene by Mouse and Rat Isolated Lung Cells. Toxicol. Sci. 51, 195-201 (1999).

Johanson, G., Ernstgard, L., Gullstrand, E., Osterman-Golkar, S., Williams, C. C. and Sumner, S. C. J. Styrene Oxide in Blood, Haemoglobin Adducts and Urinary Metabolites in Human Volunteers Exposed to C-13(8)-Styrene Vapors. Toxicology Applied Pharmacology 168: 36-49 (2000).

Kaufmann, W., Mellert, W., van Ravenzwaay, B., Landsiedel, R. and Poole, A. Effects of Styrene and its Metabolites on Different Lung Components of the Mouse – Cell Proliferation and Histomorphology. Submitted for Publication (2004).

McConnell, E.E. and Swenberg, J.A. Styrene and Styrene Oxide: Results of Studies on Carcinogenicity in Experimental Animals. In Eds M. Sorsa, Peltonen, K., Vainio, H, and Hemminki, K. Butadiene and Styrene Assessment of Health Hazard. IARC Scientific Publication No. 127: 323-334 (1993)

Nakajima, T., Elovaara, E., and Gonzalez, F. J. Styrene Metabolism by cDNA-Expressed Human Hepatic and Pulmonary Cytochrome P450. Chem Res. Toxicol. 7, 891-896 (1994).

Otteneder, E., Eder, E., and Lutz, W. K. 32P-Postlabeling Analysis of DNA Adducts of Styrene7,8 oxide at the O6 Position of Guanine. Chem. Res. Toxicol., 12, 93-99 (1999).

Otteneder, E., Lutz, U. and Lutz, WK. DNA Adducts of Styrene-7,8-Oxide in Target and Non-Target Organs for Tumor Induction in Rats and Mouse After Repeated Inhalation Exposure to Styrene. Mutation Research. 500 (1-2): 111-116 (2002).

Plopper, C. G., Hill, L.H. and Mariassy, A.T.. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung. A study of man with comparison of 15 mammalian species. Exp. Lung RESEARCH. 1, 171-180 (1980)

Roycroft, J.H., Mast, T.J., Ragan, H.A., Grumbein, S.L., Miller, R.A. and Chou, B.J. Toxicological Effects of Inhalation Exposures to Styrene in Rats ands Mice. The Toxicologist 12, 397 (1992).

Sarangapani, R., Teerguarden, J. G., Cruzan, G., Clewell, H. J., and Andersen, M. E. Pharmacokinetic Model Based Analysis of Styrene and Styrene Oxide Respiratory Dosimetry in Rodents and Humans. Inference on Dose Extrapolation and Species sensitivity. Inhalation Toxicology, 14: 789-834 (2002).

Sonich-Mullin, C., Fielder, R., Wiltse, J., Baetcke, K., Dempsey, J., Fenner-Crisp, P., Grant, D., Hartley, M., Knaap, A., Kroese, D., Mangelsdorf, I., Meek, E., Rice, J. M. and Younes, M. IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis. Reg. Toxicol. Pharmacol. 34, 146-152 (2001).

Sumner, S. C., Cruzan, G., Johanson, G., Ghanayem, B. I. and Fennell, T. R. Styrene Metabolism in Rats, Mice and Humans. CIIT Activities 21, No 3-4. 1-8 (2001).

United Kingdom, European Risk Assessment Report, Styrene, Draft, May 2002.

Vassallo, J., Curry, S., Purdon, M., Lewis, C., Fix, A. and Lehman-Keller, L. Elevated Glutathione (GSH) Pools and Gamma Glutamyl Transpeptidae (GGT) Activity in Coumarin-Induced Clara Cell Tolerance. The Toxicologist, 54. (2000).

APPENDIX 4

CALCULATION OF INHALATION EXPOSURE FROM THE USE OF LIQUID RESIN AND RESIN PASTE KITS

An initial worst case scenario must assume that such products may be used in enclosed, poorly ventilated spaces. The exposure consequences of such a worst case scenario may be calculated as follows, using the EC Algorithm and US EPA SCIES models.

EC Algorithm method

Scenario: the liquid resin product is used in a small garage and the immediate volume of air around the operator amounts to 2 m^3. No ventilation takes place and the operator stays in the concentrated atmosphere.

The EC Algorithm uses simple equations to estimate external consumer exposure.

Where:

- Amount of product used per event, q = 1 kg (assumed for car body repair work)
- Weight fraction of substance in product, wf = 0.45 (typical maximum based on industry advice)
- Fraction of substance which volatilises during duration of exposure, R = 0.1 (worst case assumption)
- Volume of air immediately surrounding the user, Vr = 2 m^3 (no ventilation)
- Ventilation rate of adult, Vinh = 1.3 m^3 /h (US EPA default for exercise such as painting)
- Duration of exposure, t = 1 h (assumed)
- Average concentration of substance in air, Cair = q x wf x R x Vr^{-1} = 22.5 g/m^3
- Amount via inhalation = Cair x Vinh x t = ~29 g/event

Results

Using the above equations and parameter values, the EC Algorithm predicts that over a one hour period a person working in this concentrated atmosphere will inhale approximately 29 g of styrene.

US EPA SCIES Program

This is US EPA computerised model for estimating the consumer exposure to chemical substances.

Scenario: the basic liquid resin scenario remains the same but SCIES requires that some additional assumptions are made. The scenario is that for latex paint. The volume of air in

which the contaminant is diluted is divided into two zones. Zone 1 represents the working area and is assumed to be a small room. However, the air within this room is expected to mix to a limited extent with the rest of the building, Zone 2. The air exchanges 0.2 times an hour. One consequence of this model structure is that, while the air around the working area may contain less styrene, the person coming away from the work area may still be exposed to a contaminant and for a much longer period, even though the work has been completed.

- Zone 1 volume = 40m^3 (default, assumed to represent a garage in this scenario)
- Whole house volume = 292 m^3 (default)
- User inhalation rate during use = 1.3 m^3/h (default)
- User inhalation rate after use = 1.1 m^3 /h (default)
- Vapour pressure = 5 torr (650 Pa at 20°C)

Results

Using SCIES, the average concentration in the work zone (Zone 1) during the period of use is 4.7 g/m^3 and the peak concentration in that zone is 7.7 g/m^3. Some exposure to lower concentrations will be maintained after the period of use. The person carrying out work is predicted to receive a total dose of approximately 20 g for one event.

In the case of resin pastes the maximum styrene concentration in the kit is 18%. This means that a lower level of styrene would be present in the air. Using the SCIES model exactly as before, with the assumptions the same, but with the weight fraction of the product as 0.18, the exposure is reduced from approximately 20 g to approximately 8 g. This lower figure will be used as an indicator for exposure arising from the use of resin pastes.

Exposure assessment based upon "bearability" of exposure

Noting that 1000 ppm will probably produce irritation and lacrimation but noting that there is no real emission data, an alternative worst case scenario may be defined in which the average concentration of styrene in the air is 8.4 g/ m^3 (double the usual bearable maximum) for the hour of use and exposure is negligible afterwards. During use of the styrene resin there is no ventilation and all of the inhaled styrene is absorbed. This produces an intake of 8.4 (concentration in g/m^3) x 1.3 (inhalation volume/hr) x 1 (time of exposure) ~11 g/event.

Results

Assuming that the airborne concentration is double the maximum bearable for one hour, with negligible exposure afterwards, the intake of styrene is ~ 11 g/event. For liquid resins this level of styrene availability in the air is theoretically possible if the product is badly misused.

APPENDIX 5

COMMENTS FROM UK INDUSTRY ON POSSIBLE SCENARIOS FOR STYRENE INHALATION DURING THE USE OF RESIN KITS

In the absence of measured data we have presented exposure estimations based upon the EC Algorithms which we feel more realistically reflect worst case consumer exposures to styrene from resin past and liquid resin kits. These cover both typical worst case uses and situations of abuse, specifically use of produce within confined unventilated spaces. Such abuse is only possible when clear warnings on product labels and literature about use of the produce in well ventilated spaces are ignored.

There is an additional need to discriminate between the use of consumers of products intended for general public and consumer use of industrial preparations that have been made available to the general public. The use of such industrial preparation by consumers or tradesmen should be seen as being as in an entirely different context to the established reinforced plastics processing industry where engineering controls can be used to reduce worker exposure and environmental emissions.

Although models of abuse situations can suggest that very high exposures might occur on the basis of the modelled assumptions, it should be realised that the likelihood of such high concentrations of styrene vapour being endured for any time is limited due to the intense odour, metallic taste, irritation and lachrymatory effects of styrene at high concentrations.

A relevant factor should you wish to take into account the possible differences in ambient temperature in various parts of Europe is that a higher temperature resin cures more rapidly so monomeric styrene is available for volatilisation for a shorter period. This reduces the loss by volatilisation and is contrary to the normal expectations for exposures to volatile materials e.g. solvents where higher temperatures might be expected to give higher atmospheric vapour concentrations.

The modelled consumer exposure in the EC Algorithm model is 22.5 g/m^3 (converts to 5,281 ppm) over a period of 1 hour. This concentration is much higher than concentrations at which nasal and eye irritation would be expected to be apparent (e.g. 375 ppm and 1 hour reported in Stewart *et al* 1968). Exposures of up to 1000 for 1 hour have been associated with severe lacrimation, increased nasal secretion, feeling of a felt – like tongue and uncertain gait {Kalsbeek *et al* 1960, cited in Malten KE and Zieluis RL, 1964 industrial Toxicology and Dermatology in the Production and Processing of Plastics Elsevier Amsterdam}. Exposure to 5,281 ppm would be a concentration that was not only severely irritating but was likely to be significantly narcotic. It is actually about the 4 hour LC50 for styrene in rats. On the basis of the irritation and the intense odour, such a concentration would not be tolerated.

The weight fraction of styrene in resin is given as 0.45. Figures of 0.40 for liquid resin or of 0.18 for resin paste would give a realistic maximum value.

The volume of air used as a basis to calculate the concentration of styrene (V_r) is presumably based upon the “Room Volume” adjusted for the immediate vicinity of the user as given within the Technical Guidance documentation. Although this is likely to give an overestimate of concentration, it may perhaps be an appropriate estimate for short exposures e.g. 3 or 6 minutes as cited in the examples in the technical guidance. However in the case of resin use over a period of an hour the styrene vapour would be expected to be dispersed throughout the volume of the working space. The mixing of the styrene vapour throughout the working space would be aided not only by diffusion and normal air movement

related to body heat convection but by the movement of the operator around the work area. (Typical work with resin paste e.g. car body repairs on the lamination of fibreglass repair is likely to involve intermittent activities in varying parts of the work area e.g. mixing, spreading/laminating and sanding/grinding).

The assumption of "no ventilation" is very extreme and equates to work within a hermetically sealed 2 m^3 box! HSE Guidance note EH22 (Ventilation of the Workplace) indicates that ventilation rates of 0.5 – 2 air changes/hour can be expected in general industrial buildings and the suggested use of a garage with a draughty up and over door would (even in still air conditions) be expected to have a natural ventilation rate of at least the upper end of this range.

No adjustment is made for the proportion of inhaled styrene absorbed via the lungs in calculation of the styrene uptake by inhalation. Studies from a number of authors suggest that 0.70 would be an appropriate proportion being absorbed (e.g. Wieczorek and Piotrowski, 1985: Int Arch Occup Environ Health 57 57-69, cited in Styrene Criteria Document ECETOC Special Report no.9).

Proposed Scenarios for Consumer Use of Unsaturated Polyester Resin Kits

(Based upon EU Technical Guidance Algorithms)

(a) Reasonable "worse case" consumer use of body filler paste: (use: filling dented bodywork on a car within a closed garage)

Assumptions: Amount of product used per event, q = 450 g (based on IK median pack size of 250 mls and product density of 1.8 g/cm^3). In such applications it is difficult to use large amounts of catalysed filler at any one time as it cures before it can be used. The need for mixing, spreading, curing and sanding to take place increases the cycle time for application of each layer of paste and consequently limits the amount of paste to be used in an hour. 450g would provide 5 x 90 g mixes of catalysed resin.

Weight fraction of styrene in-product, W_f = 0.12 (based on typical styrene concentration filler paste).

Fraction of substance that volatilises during duration of exposure R = 0.05 (based on industry laboratory work on VOC emissions which indicates 4.5% of total styrene content lost from a filler containing 12% styrene during mixing with catalyst and the cure process).

Volume of air surrounding the user, V_r = 52 m^3 (based on working in a garage of volume 35 m^3 containing a vehicle of closed volume 9 m^3) implies net workspace volume of 26 m^3. Simple arithmetical treatment would imply one air change an hour would be the equivalent of a two fold increase in diluent volume. Although the air change rate would be expected to be >=2 changes/hour, 1 air change/hour has been used to compensate for periods of inhalation of higher concentrations when working close to the source of emissions.

- Ventilation rate of adult, V_{inh} = 1.3 m^3/h (US EPA for painting work etc)
- Duration of Exposure t = 1h (likely normal maximum)

Average concentration of styrene in air, $C_{air} = q \times W_f \times R \times V_r^{-1}$

$C_{air} = 450 \times 0.12 \times 0.05/52$

$C_{air} = 52$ mg/m^3 (converts to ~ 12 ppm)

Amount of styrene absorbed in inhalation = $C_{air} = V_{inh}$ x fraction absorbed x time exposed

= 52 mg/m^3 x 1.3 x 0.7 x 1h

= 47 mg (approximately 0.6 mg/kg bodyweight for a 70 kg individual)

Comment

Using a complete 250 ml tin of resin paste within a close garage would appear even under a "worst case" assessment would give only a trivial exposure to styrene. Such an assessment is in agreement with general experience of resin kit use, particularly in light of the expected frequency of such exposures (perhaps 1-3 hours per day).

Proposed Scenarios for Consumer Use of Unsaturated Polyester Resin Kits

(Based upon EU Technical Guidance Algorithms)

(b) Reasonable "worse case" consumer use of liquid resin kit:

Assumptions:

- Amount of product used per event, q = 550 g (based on standard 500ml "consumer" pack size in UK)
- Weight fraction of styrene in product, W_f = 0.40 (based on typical maximum styrene concentration in liquid resin).
- Fraction of substance that volatilizes during duration of exposure, R = 0.075 (based on industry laboratory work on emissions from hand lay up).
- Volume of air surrounding the user V_f = 52 m^3 (based on working in a garage of volume 35 m^3 containing a vehicle of closed volume 9 m^3) implies net workspace volume of 26 m^3. Simple arithmetic treatment implies one air change an hour would be the equivalent of a two fold increase in diluent volume. Although the air change rate would be expected to be >=2 changes/hour, 1 air change/hour has been used to compensate for periods of inhalation of higher concentrations when working close to the source of emission.
- Ventilation rate of adult, V_{inh} = 1.3 m^3 /h (US EPA default for painting work etc)
- Duration of exposure t = 1 hour (likely normal maximum)

Average concentration of styrene in air, $C_{air} = q \times W_f \times R \times V_r^{-1}$

$C_{air} = 550 \times 0.4 \times 0.075/52$

$C_{air} = 318$ mg/m^3 (converts to ~ 75 ppm)

Amount of styrene absorbed in inhalation = $C_{air} = V_{inh}$ x fraction absorbed x time exposed

= 318 mg/m^3 x 1.3 x 0.7 x 1 hour

= 289 mg per event [approximately 4 mg/kg bodyweight for a 70 kg individual]

Comment

Even under worst case conditions using 500 ml of resin in a closed garage within an hour, the exposure (~75 ppm for 1 hour) would not give health concerns. Unaccustomed exposure to the distinctive odour of styrene is likely to encourage the user to seek better ventilation or fresh air. The frequency of such exposures to those purchasing consumer kits of liquid resin is unlikely to be more than a few hours/year.

Proposed Scenarios for Consumer Use of Unsaturated Polyester Resin Kits

(Based upon EU Technical Guidance Algorithms)

(c) Product abuse: worse case consumer use of body filler paste: (use within a non – ventilated car interior

Assumptions: Amount of product used per event, 1 = 450 g (based on UK median pack size of 250 mls and product density of 1.8 g/cm^3 In such applications it is difficult to use large amounts of filler at any one time as the catalysed resin cures before it can be used. The need for mixing, spreading, curing and sanding to take place increase the cycle time for application of each layer of paste and consequently limits the amount of paste to be used in an hour.

- 450 g would provide 5 x 90 g mixes of catalysed resin
- Weight fraction of styrene in product, W_f = 0.18 (based on maximum styrene concentration in filler paste)
- Fraction of substance that volatilises during duration of exposure, R = 0.05 (based on industry laboratory work on VOC emissions which indicates 4.5% of total styrene content lost from a filler containing 12% styrene during mixing with catalyst and the cure process).
- Volume of air surrounding the use, V^r = 3 m^3, (based upon typical interior volume of car passenger compartment and assuming no ventilation)
- Ventilation rate of adult, V_{inh} = 1.3 m^3 /h (US EPA default for painting work etc)

- Duration of exposure t = 1 hour (likely normal maximum, assumes that user chooses to stay within the vehicle without opening doors or windows. It might be expected that users would normally leave the vehicle to collect tools or mix resin etc).

Average concentration styrene in air, $C_{air} = q \times W_f \times R \times V_r^{-1}$

$C_{air} = 450 \times 0.18 \times 0.05/3$

$C_{air} = 1350$ mg/m^3 (converts to ~ 320 ppm)

Amount of styrene absorbed in inhalation = $C_{air} = V_{inh}$ x fraction absorbed x time exposed

= 1350 mg/m^3 x 1.3 x 1 x 1hour

= 1755 mg per event (approximately 25 mg/kg bodyweight for a 70 kg individual)

Comment

Such exposures are possible where label and other product advice is to use the product in well ventilated areas has been ignored. Whilst it is not impossible for exposure similar to that modelled in this example to occur, the likelihood that an individual will not leave the vehicle or open a car door to collect tools thus diluting the styrene content of the atmosphere etc during a period of an hour is probably low. This is particularly so as a ~320 ppm irritation and odour would be expected to prompt an individual to reduce exposure.

Should the exposure be a modelled (~320 ppm for an hour) irritation from the vapours but no actual harm would be expected.

Proposed Scenarios for Consumer Use of Unsaturated Polyester Resin Kits

(Based upon EU Technical Guidance Algorithms)

(d) Product Abuse: worst case consumer use of liquid resin kit: Repairing internal compartment of a boat with glass reinforced laminate: no ventilation)

Assumptions:

- Amount of product used per event, = 1000 g (based on maximum “consumer” pack size in UK. This would be impractical to catalyse in one operation so its use would be split into 3 or more mixing operations)
- Weight fraction of styrene in product, $W_f = 0.40$ (based on typical maximum styrene concentration in liquid resin)
- Fraction of substance that volatilizes during duration of exposure, R = 0.075 (based on industry laboratory work on emissions from hand lay up)
- Volume of air surrounding the user, $V_r = 2$ m^3

- Ventilation rate of adult, , V_{inh} = 1.3 m^3 /hour (US EPA default for paint work etc)
- Duration of exposure t = 1 hour (likely normal maximum)

Average concentration styrene in air, $C_{air} = q \times W_f \times R \times V_r^{-1}$

C_{air} = 1000 x 0.4 x 0.075/2

C_{air} = 15 g/m^3 (converts to ~ 3520 ppm)

Amount of styrene absorbed in inhalation = $C_{air} = V_{inh}$ x fraction absorbed x time exposed

= 15 g/m^3 x 1.3 x 1 x 1hour

= 19.5 g per event (approximately 280 mg/kg bodyweight for a 70 kg individual)

Comment

This is seen as being a highly unrealistic exposure scenario as such high concentrations could not be tolerated. At much lower concentrations eg 1000 ppm the irritation, lacrimation and intense odour would be expected to prompt an individual to seek fresh air.

High exposures are possible where label and other product advice to use the product in well ventilated areas has been ignored. The above scenario is very much a worst case. A confined space with no ventilation at all would be unusual as normally the route of access to the space would be expected to provide some degree of escape for the vapours by diffusion. The use of 1 kg of resin within an hour in such confined space (e.g. laminating an area of 1.5m^2 within a space with likely total surface area of perhaps 10m^2) is possible. It is however probably more likely that such an exposure would occur to other solvents where such closed compartments are painted with solvent based paints. It would therefore be seen as a general problem related to working with volatile materials within confined spaces rather than a specific problem relating to styrene based resin.

APPENDIX 6

CALCULATIONS OF DERMAL EXPOSURE FROM THE USE OF LIQUID RESIN AND RESIN PASTE KITS

EC Algorithm for Dermal Exposure

Scenario: for a worst reasonable case exposure assessment, it must be assumed that skin contact will arise through accidental spillage, or failure to wear protective equipment such as gloves during application of the resin.

Where:

- Density of product, d = 1.1 g/cm^3 (maximum for liquid resin)
- Weight fraction of substance in product, W_f = 0.45 (typical maximum)
- Thickness of layer of product in contact with skin, T_{der} = 0.1 cm (value appropriate for high viscosity liquids)
- Surface area of exposed skin, S_{der} = 280 cm^2 (estimate for the surface area of the fingers of botth hands)
- Duration and frequency of exposure are known.
- Typical maximum amount of the substance in the product, $C_{der} = d \times W_f$
- Amount of the substance on the skin, $C_{der} \times T_{der} \times S_{der}$

Results

Using the above equations, the typical maximum amount of substance in a liquid resin product, $C_{der,}$ is 0.495 g/cm^3. The amount of substance on the skin is approximately 14 g/event.

For resin pastes, with their lower maximum level of styrene, an equivalent calculation suggests a maximum value of about 5.5 g/event being available for dermal absorption.

APPENDIX 7

COMMENTS FROM UK INDUSTRY ON POSSIBLE SCENARIOS FOR DERMAL ABSORPTION OF STYRENE DURING THE USE OF RESIN KITS

In the EU algorithm calculations proposed for skin absorption the uptake via the skin is based on uptake through the total surface area of the hands. Such skin contamination should be unlikely when using resin paste as the produce is a thick paste handled with a filling knife or plastic spreading tool included in consumer kits. For liquid resin where the product is more mobile and can be applied by brush, skin absorption is more relevant consideration. It is therefore appropriate only to consider dermal absorption for liquid resin (typical density 1.1 g/cm^3 and maximum styrene content 40% w/w).

Realistic factors to take into account are:

It is very difficult to work with hands entirely covered in resin! This is particularly true when the resin is catalysed and starts to cure, as the worker's hands will stick to everything touched, such as glass reinforcement and tools. Although skin contamination may occur, gross contamination would be expected to be cleaned up without much delay for the above practical reasons. Contamination of a similarly large surface areas of skin elsewhere of the body for any period is also unlikely, apart from in cases of accidental spillage.

Based upon experimental studies on human skin the rate of absorption for liquid styrene has been measured and is relatively low (0.06 mg/cm^2/hour, Berode *et al,* 1985, Int Arch Occup Environ Health 55 331-336). The rate of absorption of styrene from catalysed liquid polyester resin would be expected to be lower than this figure for liquid styrene on account of the removal of styrene monomer from the resin as it becomes part of the matrix of the cured resin, this reducing the styrene monomer available for absorption. Conversely the rate of absorption might be expected to be higher than the liquid styrene figure as the curing resin might provide some degree of occlusion, aiding the absorption of monomer from the resin near the skin surface. On balance however, it is likely that the absorption rate for liquid styrene monomer would provide an appropriate worst case value for the absorption of styrene from a liquid resin.

The worst case scenario for dermal exposure is based upon users failing to use gloves and grossly contaminating their skin. It is relevant that liquid resin sold as consumer kits can provide as part of the kit suitable protective gloves, measuring containers and mixing implements. It is notable that the majority of consumer pack liquid resin sold by one major resin kit supplier is sold in complete kit form with accompanying gloves etc rather than as packs of resin alone. The inclusion of protective gloves within kits would be expected to encourage good working practices.

Proposed Scenarios for Consumer Use of Unsaturated Polyester Resin Kits

(Based upon EU Technical Guidance Algorithms)

Reasonable "worst case" consumer use of liquid resin kit: Dermal Exposure (use: mending damaged bodywork on a car using glass reinforced laminate within a closed garage)

- Density of liquid resin product = 1.1 g/cm^3
- Weight fraction of styrene in product, W_f = 0.40
- Thickness of layer of product in contact with skin, T_{der} = 0.1 cm (value appropriate for high viscosity materials)
- Time of exposure = 1 hour
- Surface area of exposed skin, S_{der} = 280 cm^2 (estimate for surface area of the fingers of both hands)

Using the EC algorithm concentration of styrene in product

C_{der} = 1.1 x 0.40

= 0.44 g/cm^3

Amount of substance on the skin = C_{der} x T_{der} x S_{der}

= 0.44 x 0.1 x 280

=12.3 g/event

Amount of the substance on the skin available for uptake = 90% of the amount on the skin (10% evaporates) =12.3 x 0.9

=11g/event

Comment

In normal use whilst some accident skin exposure may occur due to lack of skin protection and product spillage is unlikely that the total surface area of hands will be covered. Should gross contamination occur due to significant accident spillage etc then removal of excess resin before continuing work might be expected for practical reasons. Therefore the surface area of the fingers of both hands has been considered to be a more appropriate estimate than the total area of both hands.

APPENDIX 8
CALCULATION OF DAILY DIETARY INTAKE OF STYRENE

Food Group	Styrene mean levels (μg/kg)		Consumption (kg/person/day)	Daily dietary styrene intake (μg/person/day)	
	Lower bound	Upper bound		Lower bound	Upper bound
Bread	0.7	1.1	0.108	0.076	0.12
Miscellaneous cereals	1.2	1.2	0.101	0.12	0.12
Carcass meat	0.7	1.1	0.022	0.015	0.024
Offal	3.4	3.4	0.001	0.003	0.003
Meat products	3	3	0.047	0.14	0.14
Poultry	3.4	3.4	0.019	0.065	0.065
Fish	4.8	4.8	0.014	0.067	0.067
Oils & fats	7.9	7.9	0.027	0.213	0.213
Eggs	2.9	2.9	0.014	0.041	0.041
Sugars & preserves	1.6	1.8	0.063	0.101	0.113
Green vegetables	0.2	0.7	0.034	0.007	0.024
Potatoes	0	0.3	0.123	0	0.037
Other vegetables	0.6	1.1	0.076	0.046	0.084
Canned Vegetables	1.3	1.6	0.033	0.043	0.053
Fresh fruit	0.1	0.4	0.069	0.007	0.028
Fruit products	3.1	3.4	0.044	0.136	0.150
Beverages	0.8	1.2	0.996	0.797	1.195
Milk	0.2	0.7	0.281	0.056	0.197
Dairy products	2.4	2.4	0.06	0.144	0.144

Nuts	8.7	8.7	0.002	0.017	0.017
Total				2.1	2.8

ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union

EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GC	Gas chromatography
GC-ECD	Gas chromatography with an electron capture detector
GC-ECNI-HRMS	Gas chromatography with electron capture negative ion high resolution mass spectrometry
GC-EI-MS	Gas chromatography with electron impact mass spectrometry
GC-FID	Gas chromatography with a flame ionisation detector
GC-HRMS	Gas chromatography with high resolution mass spectrometry
GC-LRMS	Gas chromatography with low resolution mass spectrometry
GC-MS	Gas chromatography with a mass spectrometry detector
GC-NCI-MS	Gas chromatography with negative ion chemical ionisation mass spectrometry
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MS	Mass spectrometry
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TLC	Thin layer chromatography
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative

v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)