Technical Report

No 52

Styrene Toxicology Investigations on the Potential for Carcinogenicity

November 1992

ISSN-0773-8072-52

TECHNICAL REPORT

No.52

Technical Report No. 52

STYRENE TOXICOLOGY

INVESTIGATIONS ON THE POTENTIAL FOR

CARCINOGENICITY

November 1992

ISSN-0773-8072-95

Brussels, September 1992

ECETOC Technical Report No. 52

STYRENE TOXICOLOGY INVESTIGATIONS ON THE POTENTIAL FOR CARCINOGENICITY
CONTENTS

STYRENE TOXICOLOGY INVESTIGATIONS ON THE POTENTIAL FOR CARCINOGENICITY

SUMMARY

At present there is no clear evidence that styrene is carcinogenic in laboratory animals or in man. The absence of an exposure related oncogenic response in several animal bioassays or appropriate epidemiology investigations precludes the use of quantitative risk assessment procedures. Concern has been expressed, however, that a putative carcinogenic potential exists as a result of the formation and presence in tissues of styrene 7,8-oxide, an intermediate metabolite of styrene.

To address these concerns new toxicokinetic data on styrene and the metabolic occurrence of styrene oxide have been generated and are presented together with information on macromolecular binding in vivo. The toxicokinetic parameters for styrene and styrene oxide permit a quantitative description of the major pathways of styrene and styrene oxide in the rat and mouse under various conditions of exposure.

Based on experimental data a physiologically based pharamcokinetic (PB-PK) model for styrene and styrene oxide has been developed, validated and used to calculate the body burden of styrene and styrene oxide for man in relation to animals in terms of AUC_{SO} (AUC_{SO} is the area under the concentration time curve for styrene oxide in blood). This approach referred to as "semiquantitative margin of safety" ("SMS") provides the opportunity to assess the putative hazard of styrene to man in relation to specific conditions in laboratory animals. Comparison of predicted and measured AUC_{so} values in animals with that in man exposed to an anticipated workplace concentration of 50ppm styrene shows AUC_{SO} values up to 400 times lower in man than in exposed animals in which no treatment related oncogenic response was observed.

In addition, the results of DNA binding studies showed that styrene metabolism in the rat or the mouse does not lead to the production of biologically significant DNA binding intermediates. Studies with styrene oxide failed to find any evidence of DNA binding while investigation with styrene showed only minute levels of binding considered too low to cause an increase in the incidence of cancer.

It can be concluded that the carcinogenic potential of styrene, if one exists at all, must be so low that occupational or environmental exposure to styrene is unlikely to present any genotoxic or carcinogenic hazard for man.

SECTION 1. INTRODUCTION

Styrene is an important chemical of wide industrial use, particularly in the manufacture of polymers and reinforced plastics. Styrene is a naturally occurring as well as a man-made chemical. Entry into the atmosphere occurs via automobile exhaust, as a result of smoking, during production, storage and processing of styrene, and to a minor extent during or after use of the final product. Environmental and occupational exposures to styrene occur predominantly via inhalation. The toxicity of styrene has been reviewed (Fielder, 1981; WHO, 1983; BUA, 1990; Vainio, 1991). Irritation of the mucous membranes and effects on the nervous system are the most commonly reported occupational findings.

In recent years the discussion about a possible carcinogenic potential of styrene has become controversial. At present, there is no clear evidence that styrene is carcinogenic in man or laboratory animals; the available data are inadequate to reach definitive conclusions. Limitations and deficiencies exist in many of the long-term animal studies and human epidemiology studies involving styrene. The toxicological significance of styrene-7,8-oxide (styrene oxide) as an intermediate metabolite of styrene has become the focus of recent concern. Styrene oxide is mutagenic in *in vitro* prokaryotic and eukaryotic systems (Appendix D.1). In long-term gavage studies it causes increased tumour incidences in the forestomach of rodents at high doses but no treatment related systemic tumours were detected (Appendix C.3). The significance of these tumours is highly uncertain with respect to risk estimation for man where styrene oxide occurs only at low concentrations as an intermediate metabolite.

Knowledge of the kinetics of formation and degradation of styrene oxide together with definitive data on the macromolecular binding of styrene and styrene oxide are important prerequisits for understanding tissue dosimetry. The use of such data in a physiologically-based pharmacokinetic model (PB-PK) provides a better understanding of the internal dosimetry of styrene oxide in laboratory animals and man allowing a more meaningful evaluation of the carcinogenic potential of styrene than was formerly possible.

The objective of this document is to present new information on metabolism, toxicokinetics and macromolecular binding as generated in the ECETOC research programme which substantially contribute to the evaluation of the carcinogenic potential of styrene.

The following studies were performed:

- Study on the kinetics of styrene and styrene oxide in rats and mice.

Dr. J. G. Filser (1992). Forschungszentrum fuer Umwelt und Gesundheit GmbH, Institut fuer Toxikologie, 8000 Muenchen.

- Investigation of the adduct formation between styrene or styrene-7,8-oxide and deoxyribonucleic acid (DNA) in rats, in mice and *in vitro*.
  Prof. Dr. W. K. Lutz (1992). Eidgenoessische Technische Hochschule und Institut fuer Toxikologie, Universitaet Zuerich.

- Investigation of the adduct formation between styrene or styrene metabolites and haemoglobin or blood proteins in rats and mice (*in vitro* and *in vivo*).
  Dr. S. Osterman-Golkar (1992). Dep. of Radiobiology, University Stockholm.

The above reports are available from ECETOC.

Data from these studies are used to examine the putative hazard of styrene for man in relation to specific conditions used in laboratory animal studies.

SECTION 2. PRESENTATION OF NEW RESULTS

2.1. TOXICOKINETICS

Concern about the carcinogenic potential of styrene has been focused on the occurrence of styrene oxide as an intermediate metabolite of styrene (Appendix A). Previous studies suggested that the rat is a good animal model for understanding the toxicokinetics of styrene in man, at least for inhalation exposure concentrations up to 80ppm. Anderson and Ramsey (1983) developed a (PB-PK) model which describes the non-linear behaviour of styrene in rats following higher exposures (Appendix A.4). Toxicokinetic data for styrene oxide and its relation to styrene had not however been determined. Although scattered analytical data on styrene oxide in blood are available, these are inadequate for making predictions regarding body burden of styrene oxide in rats or man exposed to styrene. Furthermore kinetic data for styrene in mice (inhalation) are not available. The studies of Filser (1992) were performed in the ECETOC research program to fill these data gaps period.

The results of these studies show that at steady state the rate of metabolism of inhaled styrene in rats and mice increases linearly with exposure concentration up to about 260ppm. Below this concentration, there is little bioaccumulation of inhaled styrene. At concentrations below 260ppm transport to the metabolising enzymes is the rate limiting step for metabolism rather than their enzymatic capacity. Kinetic behaviour of styrene is strongly influenced by physiological parameters such as blood flow and especially alveolar ventilation rate.

At exposure concentrations of styrene above 300ppm the rate of metabolism at steady state is limited by biochemical parameters of the metabolizing enzymes (V_{max} and Km_{app}). Saturation of metabolism (V_{max}) is reached at about 600ppm in rats and 900ppm in mice (rats: 224µmol/hrxkg; mice: 625µmol/hrxkg). Above 300ppm, bioaccumulation increases rapidly with increasing styrene exposure. At levels above 2,000ppm, bioaccumulation reaches its maximum.

The concentration of styrene oxide was measured in the blood of rats and mice following exposure to styrene or styrene oxide via different dose routes (oral, intraperitoneal and inhalation). At steady state following exposures to styrene between 20 and 800ppm, styrene oxide was detected in the blood of both species. In rats, blood concentrations of styrene oxide correlate linearly with the rate of metabolism of styrene. This indicates that the rate-limiting step is the formation of styrene oxide rather than its detoxification. In contrast, such a clear correlation is not found in mice. At exposure concentrations up to 260ppm similar

styrene oxide kinetics are seen in both species. A sharp increase of styrene oxide levels occurs in mice after exposure to styrene concentrations higher than 260ppm.

The latter observation is interpreted as follows: at exposure concentrations of styrene below 260ppm the relatively higher metabolic rate (styrene to styrene oxide) in mice is compensated by a high elimination rate of styrene oxide. This leads to comparable amounts of styrene oxide in rats and mice. At exposure concentrations above 260ppm, greater amounts of styrene oxide become systemically available in mice than in rats. In mice, levels up to 7,500ng/ml were reached during exposure to 800ppm styrene, while in rats blood concentrations of styrene oxide did not exceed 460ng/ml. This may indicate that certain styrene oxide metabolic pathways are overwhelmed in the mouse at styrene concentrations greater than 260ppm.

Concentrations of styrene oxide in blood after intraperitoneal or intravenous administration of styrene oxide to rats and mice confirmed the results of the inhalation experiment with styrene, indicating that biotransformation of styrene oxide is faster in mice than in rats at low styrene exposure concentrations.

Concentrations of styrene oxide in blood were also measured after oral administration of styrene oxide to rats and mice. In both species a large variation of blood/plasma styrene oxide concentrations was found. Despite this variation it could be concluded that styrene oxide in blood after oral administration represented <5% of that seen after i.p. administration of the same dose. This difference may, at least partly, be due to the fast hydrolysis of orally administered styrene oxide at the acidic pH in the stomach.

Based on the new experimental data, a physiologically-based pharmacokinetic model (PB-PK) has been developed and verified by Filser and Nolan (in press). Body burden or tissue levels in rats or mice can now be predicted for various conditions of exposure to styrene or styrene oxide. This model can also be used to predict the styrene oxide body burden for man exposed to styrene. Such calculated/predicted results have been verified by experimental data.

2.2. MACROMOLECULAR BINDING

A review of published data concerning DNA and haemoglobin adduct formation is given in Appendix B.

2.2.1. HAEMOGLOBIN BINDING

As part of the ECETOC research programme the study conducted by Osterman-Golkar (1992) investigated the possible adduct formation between styrene or styrene oxide and haemoglobin or other blood proteins in rats and mice following intraperitoneal (i.p.) administration of styrene or styrene oxide and to determine the feasibility of *in vivo* monitoring of styrene exposure by quantification of blood protein adducts. A summary of results is presented below.

The experiments show that haemoglobin adducts are formed and can be detected following i.p. administration of styrene or styrene oxide to rodents. Methods were developed for the determination of an N-terminal valine adduct (hydroxyphenethylvaline); this adduct was found to be stable in contrast to adducts involving carboxyl groups which were found to be unstable. The amount of N-terminal valine adducts, as a measure of *in vivo* dose was determined in mice and rats administered styrene oxide or styrene at doses ranging up to about 250mg/kg body weight.

In mice treated over a dose range of 50 to 250mg styrene oxide/kgbw the dose response curve for styrene oxide binding to N-terminal valine is non-linear with a disproportionate increase in binding being seen at the higher dose levels. This effect is consistent with toxicokinetic data which shows that at high systemic SO exposures the detoxification system in the mouse becomes overwhelmed thereby compromising the clearance mechanism. In mice dosed with styrene the level of binding was considerably lower than that seen in mice treated with an equivalent dose of the oxide, e.g. the level of binding measured in mice treated with styrene (dose level approximately 200mg/kgbw) was only about 5% of that measured in mice receiving an equivalent dose of the oxide.

Similar studies in the rat also indicated a deviation from linearity with again comparatively more binding being seen with increasing dose. In the rat however, this deviation was much smaller as compared with the mouse; again supporting the toxicokinetic data that had shown that the rat is more efficient at clearing SO from the body than is the mouse. The levels of binding measured in the rat were also lower as compared with the mouse, i.e. at an i.p. dose of approx. 50mg/kgbw the level of N-terminal valine binding was approximately 3 times higher in the mouse as compared with rat.

With further refinement and verification by the inhalation dose route, measurement of haemoglobin adducts may provide the basis for the development of a suitable biological monitoring method.

2.2.2. DNA BINDING

In long-term gavage studies styrene oxide has caused an increased incidence of tumours in the forestomach of rodents at high doses, but no increase in systemic tumours (Appendix C.3). Genotoxic and non-genotoxic mechanisms may be involved in the development of the tumours. To assist in the evaluation of the genotoxic potential of styrene oxide, DNA binding experiments were conducted *in vivo*. A summary of the results generated for ECETOC by Lutz (1992) is presented below.

In vitro experiments showed that DNA adducts were formed to a small but measurable extent during a 24-hour incubation of tritiated styrene oxide with calf thymus DNA. This is consistent with the *in vitro* mutagenic potential of styrene oxide (Barale, 1991).

To evaluate the genotoxic potential of styrene oxide *in vivo*, radiolabelled styrene oxide was administered by gavage in corn oil to male CD rats at levels of 1.65 and 240mg/kgbw. After 4 or 24 hours, DNA from forestomach, glandular stomach and liver was isolated, purified and its radioactivity determined. At 4-hours the radioactivity in DNA samples from the forestomach and the liver at both dose levels was below the limit of detection. Expressed in units of the Covalent Binding Index, [CBI = μmol adduct per mol DNA nucleotide/mmol chemical administered per kg body weight; Lutz, 1986], the DNA-binding potency in the forestomach and liver was below 2.6 and 2.0, respectively. In the glandular stomach at 4 hours and in most 24 hour samples, DNA was slightly radiolabelled. Enzymatic degradation of the DNA and separation of the nucleotides showed that the tritium represented biosynthetic incorporation of radio-label into newly synthesized DNA rather than covalently bound radiolabel. The limit of detection for DNA adducts in the glandular stomach was at a CBI <1.0.

Radiolabelled styrene oxide was administered by i.p. injection to male B6C3F1 mice. Liver DNA was analysed after 2 hours. No radioactivity was detectable at a limit of detection of CBI <0.6.

Although forestomach tumours were observed in bioassays (Maltoni *et al*, 1979), the *in vivo* studies with styrene oxide in rats did not demonstrate detectable DNA adducts in the forestomach at a limit of detection of CBI <2.6. This corresponds to an ability to detect 7 adducts per 10^8 DNA nucleotides in the low dose experiment. Hence a purely genotoxic mechanism of tumorigenic action of styrene oxide in the forestomach is unlikely.

Radiolabelled styrene was also administered by inhalation in a closed chamber to male and female CD rats and B6C3F1 mice. The metabolised dose was between 20 and

39mg/kgbw in rats and between 70 and 110mg/kgbw in mice. Exposure time was between 5 and 9 hours and peak concentrations in the chamber were at 200 and 400ppm, for rats and mice, respectively. DNA from the liver and the lung (rats only) was purified and analyzed for nucleotide adducts. In mouse liver DNA a small but detectable amount of radioactivity eluted with similar retention times as adducts prepared from calf thymus DNA and styrene oxide *in vitro*. The DNA-binding potency averaged at 0.1CBI units.

In rat liver DNA, no adducts were observed at a limit of detection of 0.1 to 0.2CBI units. Two DNA samples from rat lung available at high yield allowed a lower limit of detection. Adduct-related radioactivity was detectable at the level of 0.07CBI units.

The results of these experiments indicate that styrene metabolism in the rat and mouse does not result in the production of potent DNA binding intermediates. This conclusion is supported by the studies with styrene oxide in which no adduct formation was detected in these species.

SECTION 3. COMPARATIVE EVALUATION OF DATA

3.1. EVALUATION OF DATA IN RELATION TO BIOASSAYS

The assessment of hazard from exposure to chemicals focuses on dose-related adverse effects seen in animal bioassays or epidemiological investigations. Quantitative carcinogenicity risk assessment procedures require an exposure related oncogenic response in either laboratory animals or in human beings. Such approaches are not possible with styrene using the carcinogenicity data currently available. Eleven long-term animal studies with styrene to date have collectively shown no clear evidence of carcinogenicity related to styrene (Appendix C.2). Likewise the available epidemiology information shows no convincing evidence of cancer in workers occupationally exposed to styrene. Hence, there is no basis to conclude that styrene is carcinogenic.

Despite this, there is some concern about the putative carcinogenic potential of styrene since styrene oxide is an intermediate metabolite of styrene and is positive in *in vitro* mutagenicity assays. Styrene oxide has also been shown to cause tumours of the forestomach in rodents when given at high oral doses by gavage (Appendix C.3). The neoplasias were associated with chronic irritation of the forestomach and there was no treatment-related systemic oncogenic effect. Moreover, there was no evidence of carcinogenicity at the site of application when styrene oxide was applied repeatedly to the skin of rodents.

The oncogenic response to styrene oxide in the rodent forestomach after gavage treatment is inappropriate as a basis for making a quantitative risk assessment for human beings exposed to styrene by inhalation. The relevance of rodent forestomach tumours for human risk is quite controversial.

Although conventional approaches to quantitative risk assessment are not possible for styrene, biochemical/mechanistic data are useful in clarifying the role of styrene oxide as intermediate metabolite and may help to answer concerns about its occurrence upon styrene exposure. Such considerations require an understanding of numerous factors, including species differences in the relative rates of formation and degradation of styrene oxide in various organs and tissues. The physiologically-based pharmacokinetic (PB-PK) model developed by Filser and Nolan (in press) and validated with the above data (section 2) provides a useful tool for these comparisons. The subsequent evaluation is therefore based on the experimental data and the PB-PK model comparing the internal styrene oxide dosimetry (AUC_{SO}) in the blood of laboratory animals and man under various exposure conditions to styrene and styrene oxide. Thus a perspective on the differences in tissue

styrene oxide levels in man occupationally exposed to styrene and in rodents showing no or minimal responses following exposure to styrene or styrene oxide is provided. In using this approach it is assumed that human or animal tissues show similar biological responses with comparable doses of styrene oxide. Since the area under the blood concentration time curve (AUC) is the integral of the toxicokinetic behaviour of a chemical, this parameter rather than peak blood levels has been used as surrogate for tissue dose. These AUC_{SO} values will then be used to examine the putative hazard of styrene for man. In this document this will be referred to as a "semiquantitative margin of safety" ("SMS").

This basic concept of a qualitative approach to risk assessment has recently been adopted by the "Committee on Carcinogenicity" in the Department of Health (HSE; 1991).

3.1.1. STYRENE OXIDE DOSIMETRY (AUC_{SO}) IN MAN EXPOSED TO STYRENE

Studies reported by Lof *et al* (1986a) indicated that maximum styrene oxide blood levels were of the order of 3.0×10^{-5}mmol/l when measured in human volunteers exposed to 70ppm styrene. This value is comparable with the maximum predicted styrene oxide blood concentration of 2.8×10^{-5}mol/l derived by using the PB-PK model (Figure 1). The close agreement between the measured and predicted values indicates that the PB-PK model is a reliable tool for estimating blood styrene oxide concentrations in man exposed to styrene.

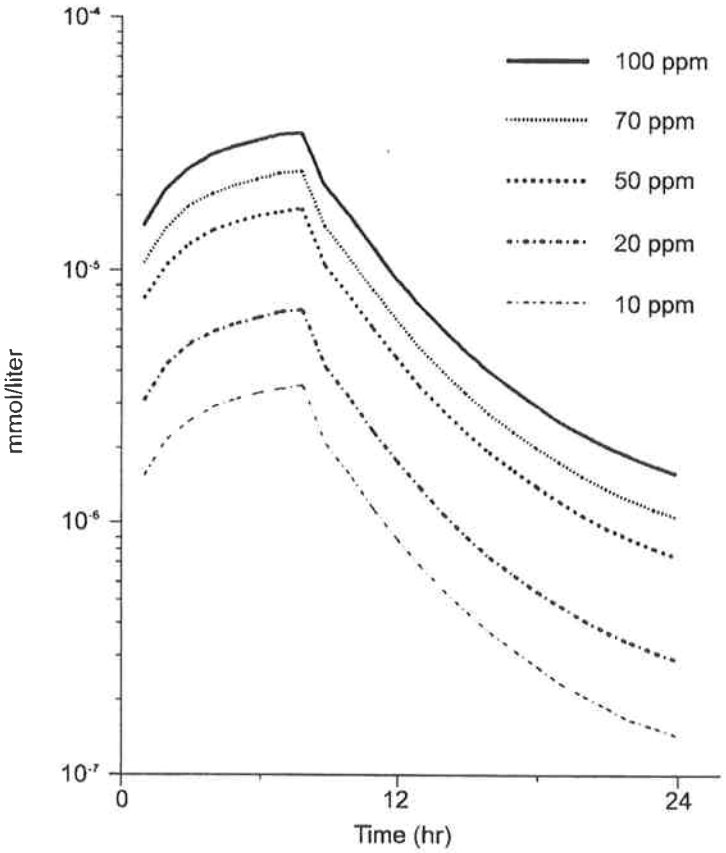
The predicted blood styrene oxide concentrations in human beings exposed for 8 hours to 10, 20, 50, 70 or 100ppm styrene are shown in Figure 1, and the resulting AUC_{SO} values in man at these styrene exposure concentrations are compiled in Table 1. The predicted AUC_{SO} values increase linearly over a styrene exposure concentration range of 10 to 100ppm, as indicated by an approximate 10-fold increase in AUC_{SO} values with the 10-fold increase in styrene exposure concentration. National occupational exposure standards for styrene currently range from 10ppm to 100ppm. For the purpose of estimating the "SMS" values, a mid-range value of 50ppm has been used for man.

3.1.2. STYRENE OXIDE DOSIMETRY (AUC_{SO}) IN RATS AND MICE FOLLOWING ORAL ADMINISTRATION OF STYRENE OXIDE

In the bioassay reported by Lijinsky (1986) male and female Fischer 344 rats were given 275 or 550mg/kgbw, and B6C3F1 mice were given 375 or 750mg/kgbw styrene oxide in corn oil, 3 times per week, for up to 104 weeks. In mice, the high dose exceeded the maximum tolerated dose (MTD), as indicated by high levels of mortality. Consistent with the results of other styrene oxide bioassays (Appendix C.3), a high, dose-related

FIGURE 1

Predicted concentrations of styrene oxide in the blood of humans exposed for 8 hours to various concentrations of styrene.



incidence of squamous cell carcinomas and papillomas, as well as other non-neoplastic lesions were found in the forestomach of the styrene oxide-treated animals. There was no dose-related increase in tumours in any other organ or tissue in either male or female rats or mice. The AUC_{SO} resulting from the high oral styrene oxide doses given in these studies were not associated with any systemic oncogenic response in either rats or mice. Using the same dose regime 275 or 550mg/kgbw styrene oxide by gavage in corn oil, Langvardt and Nolan (1991) demonstrated that the average AUC_{SO} values were 783 and 4,768µg x hr/kg, respectively, in male Fischer 344 rats. The corresponding experimentally determined blood styrene oxide time-course is shown in Figure 2. Therefore, the absence of a systemic oncogenic response in the styrene oxide bioassays cannot be attributed to a lack of systemic exposure to styrene oxide. The PB-PK model of Filser and Nolan (in press) predicts AUC_{SO} values of 1,069 and 2.320µgxhr/kg, respectively, in rats for the

TABLE 1
PREDICTED STYRENE OXIDE BLOOD CONCENTRATION (AUC_{SO}) COMPARISONS

Species	Exposure (Dose)	Route	AUC_{SO} (µg x hr/kg)
Human	10ppm Styrene	Inhalation	3.8
Human	20ppm Styrene	Inhalation	7.6
Human	50ppm Styrene	Inhalation	19.2
Human	70ppm Styrene	Inhalation	27.0
Human	100ppm Styrene	Inhalation	38.8
Rat	275mg/kgbw SO[a]	Oral	1,069
Rat	550mg/kgbw SO[a]	Oral	2,320
Rat	500mg/kgbw Styrene	Oral	4,968
Rat	1,000mg/kgbw Styrene	Oral	7,931
Rat	2,000mg/kgbw Styrene	Oral	11,591
Mouse	150mg/kgbw Styrene	Oral	994
Mouse	300mg/kgbw Styrene	Oral	2,310
Rat	600ppm Styrene	Inhalation	2,534
Rat	1,000ppm Styrene	Inhalation	3,980

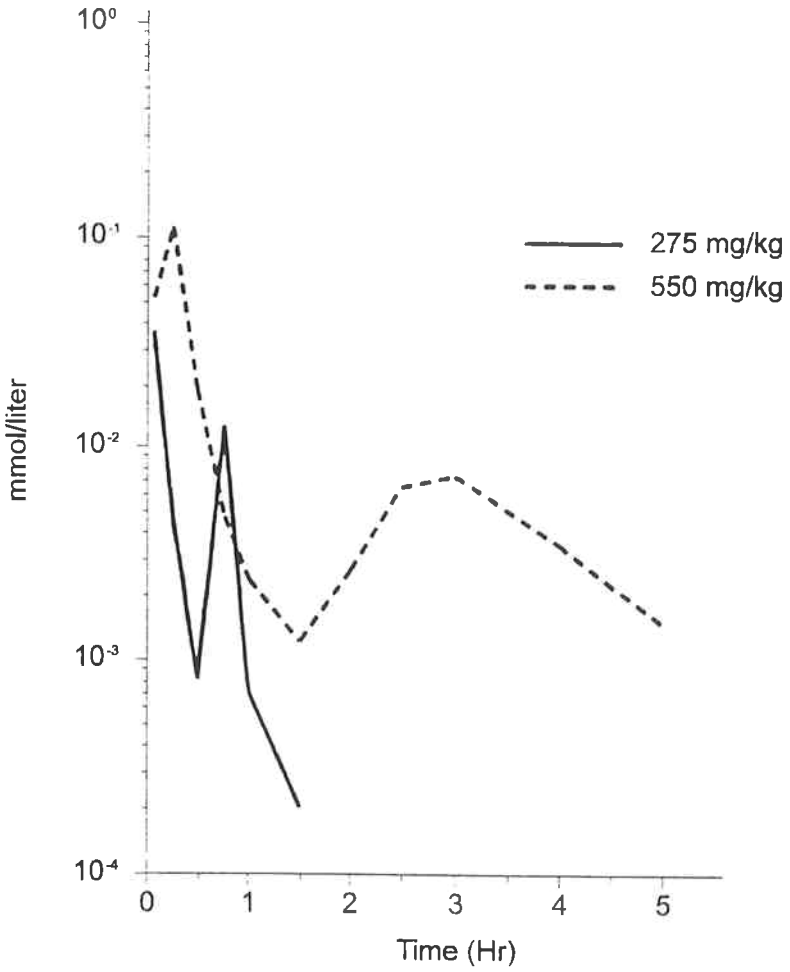
[a]Styrene-7,8-oxide

same dose levels: 275 and 550mg/kgbw styrene oxide by gavage (see Table 1).

Comparison of the predicted AUC_{SO} value at 550mg/kgbw with the predicted AUC_{SO} in man exposed for 8 hour to 50ppm styrene (19,2µgxhr/kg) shows that the SMS value is approximately 120 for human beings in relation to the (highest dose - 550mg/kgbw) AUC_{SO} in rodent oral styrene oxide bioassays in which no systemic oncogenic response was found.

FIGURE 2

Observed concentrations of styrene oxide in the blood of rats given an oral dose of 275 or 550mg/kg styrene oxide



3.1.3. STYRENE OXIDE DOSIMETRY (AUC_{so}) IN RATS AND MICE FOLLOWING ORAL ADMINISTRATION OF STYRENE

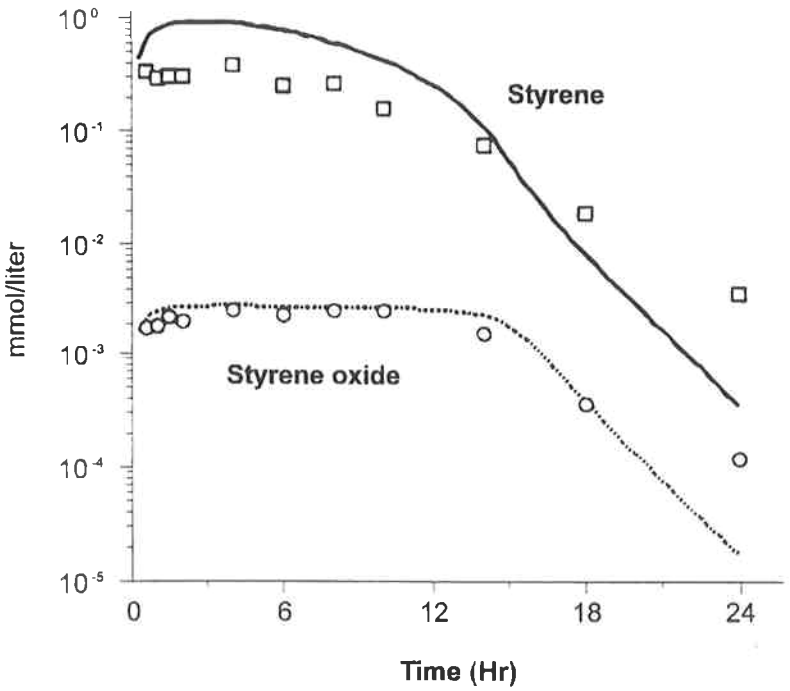
In the NCI (1979) styrene bioassay, male and female Fischer 344 rats were given oral doses of 0, 500, 1,000 or 2,000mg/kgbw, and B6C3F1 mice were given 0, 150 or 300mg/kgbw styrene in corn oil, 5 days/week, for 78 weeks (Appendix C.2). The 2,000mg/kgbw dose level exceeded the MTD as indicated by excessive treatment-related mortality; survival of rats at this dose level was inadequate to evaluate potentially late developing tumours. There was also a dose-related increase in mortality in male mice given styrene at the dose of 300mg/kgbw. The authors of the NCI bioassay concluded

that there was no clear evidence of treatment-related carcinogenicity in either male of female rats or mice.

To simulate these study conditions Mendrala *et al* (1992) measured blood styrene oxide concentrations in naive and pre-exposed Sprague-Dawley rats given an oral dose of 500mg/kgbw styrene in corn oil (Appendix A.4). The mean blood styrene oxide concentrations ranged from 0.07 to 0.53µg/g during the first 10 hours after dosing and the AUC_{SO} was calculated to be 3,320µgxhr/kg (Figure 3). Repeated exposure to styrene did not significantly alter blood styrene oxide levels or the AUC_{SO}.

FIGURE 3

Observed and predicted concentrations of styrene and styrene oxide in rats given a 500mg/kg oral dose of styrene. Lines depict predictyd concentrations and symbols depict the observed concentrations.

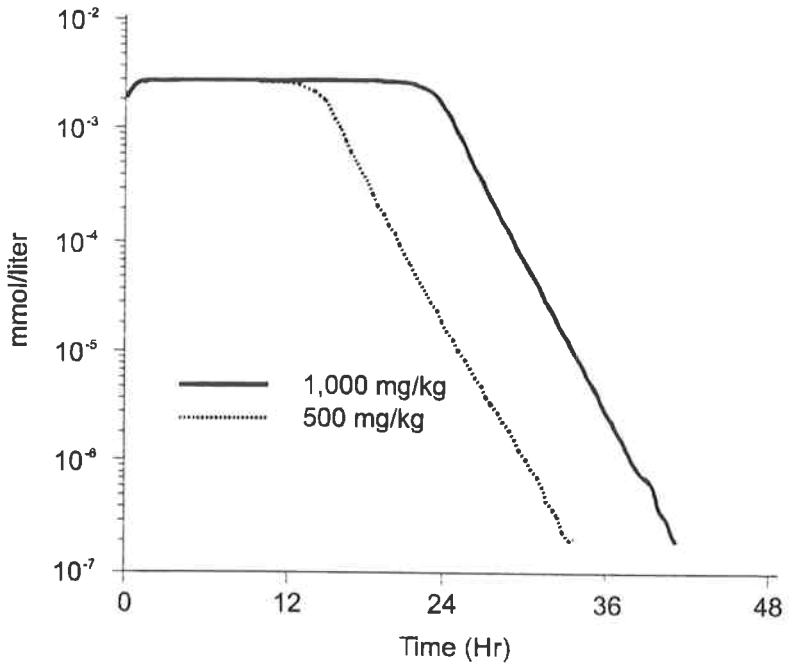


The PB-PK model (Filser and Nolan, in press) predicts that, the AUC_{SO} in rats given an oral dose of 500mg/kgbw styrene is 4,968µgxhr/kg (Figure 4; Table 1). The good agreement between the predicted and experimentally determined AUC_{SO}'s supports the value of the PB-PK model. For rats given 1,000mg/kgbw styrene orally, the predicted AUC_{SO} is 7,937µgxhr/kg (Figure 4; Table 1). For mice, the predicted AUC_{SO} values are

994 and 2,310µgxhr/kg for oral styrene doses of 150 and 300mg/kgbw, respectively (Figure 5; Table 1). Comparison of these predicted AUC_{so} values with those of man exposed to 50ppm styrene (Table 1) shows that the "SMS" value is greater than 400 for man relative to AUC_{so}'s that were not associated with an oncogenic response in rodents given high oral doses of styrene.

FIGURE 4

Predicted time course of styrene oxide in blood of rats given oral doses of 500 and 1,000mg/kg styrene.



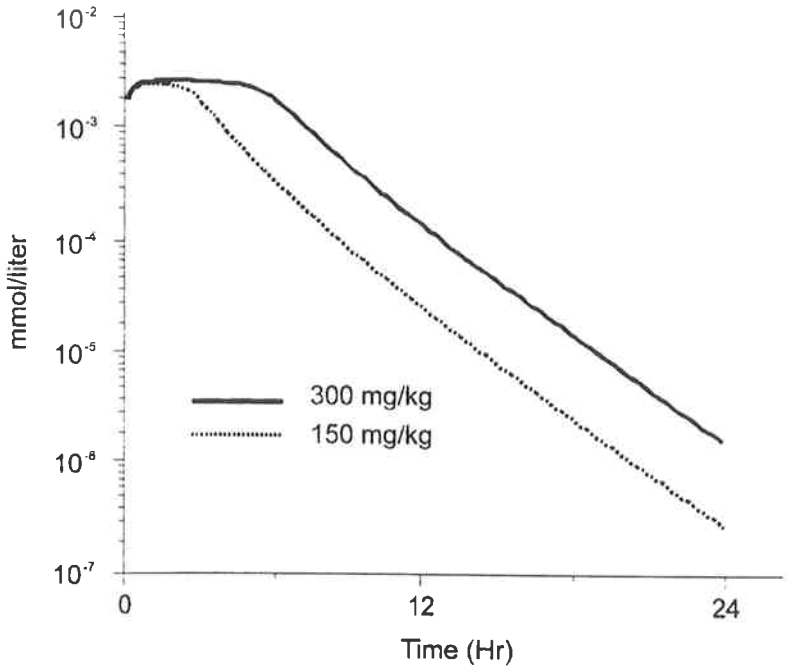
3.1.4. STYRENE OXIDE DOSIMETRY (AUC_{so}) IN RATS FOLLOWING INHALATION OF STYRENE

In the study reported by Jersey *et al* (1978), male and female Sprague-Dawley rats were exposed by inhalation to 0, 600 or 1,000ppm styrene 6hrs/day, 5 days/week, for up to 2 years (Appendix C.2). Interpretation of the results of this study was complicated by the outbreak of chronic murine pneumonia unrelated to the styrene exposures but there was no conclusive evidence of a treatment related oncogenic response.

The predicted AUC_{so} in rats exposed to 1,000ppm styrene vapours is 3,980µg x hr/kg (Figure 6; Table 1). Comparison of this predicted AUC_{so} with that of man exposed to

FIGURE 5

Predicted time course of styrene oxide in blood of mice given oral doses of 150 and 300mg/kg styrene.



50ppm styrene (Table 1) shows that the "SMS" value is approximately 200 for man in relation to the rat AUC_{SO} that was not conclusively associated with an exposure related oncogenic response.

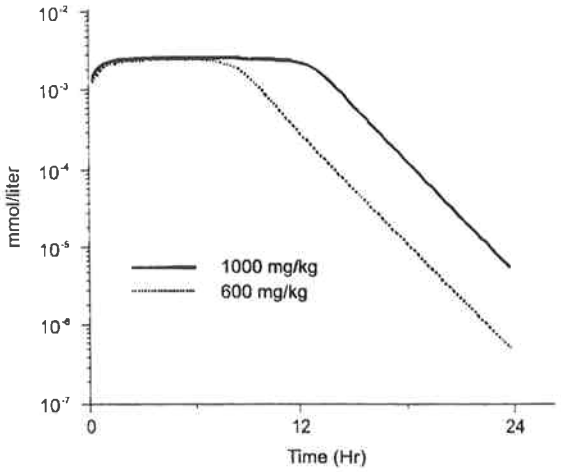
3.2. EVALUATION OF MACROMOLECULAR BINDING DATA

3.2.1. HAEMOGLOBIN BINDING STUDIES

Covalent binding of biological active compounds to haemoglobin has been proposed for biological monitoring. Although covalent binding to proteins indicates chemical reactivity (alkylation, arylation, etc.), it cannot be used as an indicator of DNA binding or other genotoxic effects unless a correlation can established between the two. Data reported here and published data (Osterman-Golkar, 1991 and 1992) show that non-specific reactions between styrene oxide and blood proteins can occur. However, the adduct of styrene oxide with the N-terminal valine of haemoglobin can be used as being an indicator of styrene or styrene oxide exposure in rodents. While it is possible that with further refinement this method could be used for monitoring human exposure no conclusions can

FIGURE 6

Predicted time course of styrene oxide in blood of rats exposed for 6hr to 1,000 and 600ppm styrene.



be made for the evaluation of the carcinogenic potential of styrene or styrene oxide, due to the lack of data on correlations with DNA-binding.

3.2.2. STYRENE AND STYRENE OXIDE DNA-BINDING STUDIES

DNA binding studies have been conducted *in vitro* and *in vivo* with styrene and styrene oxide (Lutz and Cantoreggi, in press). In vitro experiments with styrene oxide showed a small but detectable binding to calf thymus DNA; this observation is consistent with the weak in vitro mutagenic potency of styrene oxide. Studies *in vivo* did not detect DNA adducts in the forestomach of rats administered styrene oxide orally or in the livers of mice receiving styrene oxide by i.p. injection. These findings are consistent with the hypothesis that the forestomach tumours seen in styrene oxide rodent bioassays do not result from a purely genotoxic mechanism.

Following inhalation of styrene, a small but detectable amount of DNA binding occurred in mouse liver whereas in rat lung and liver no binding was found at the limit of detection of this assay.

Comparison of these data with findings in other substances can provide a perspective on the possible carcinogenic potential of styrene. The covalent binding index (CBI) has been used for making these comparisons, since it has been shown that there is a relatively

good correlation (r = 0.81) between carcinogenic potency and liver CBI values for 29 activation-dependent mutagenic carcinogens (Lutz, 1986). The CBI values for these 29 substances range from 2 to 10,000 and the carcinogenic potencies (TD_{50} values) range from $1x10^{-6}$ to 8mmol/kg/day. If this correlation holds for the DNA binding potency of styrene (CBI = 0.1), a TD_{50} value of about 1,000mmol/kg/day (100g/kg/day) of styrene is calculated.

The above comparison cannot be made for styrene oxide since this concept is only applicable to activation dependent carcinogens.

Further information about DNA binding of styrene or styrene oxide can be expected from the use of the ^{32}P post-labelling technique which in principle should have a detection limit 1-2 orders of magnitude lower than the $^{3}H/^{14}C$ radiolabel method. However, the former method requires extensive basic investigations on each test compound prior to the test itself. Such experiments are currently being carried out as a prerequisite for future tests (Hemminki *et al*, 1990; Lutz, 1992; Pongracz *et al*, 1992); previously published data from ^{32}P post-labelling analysis of DNA samples from styrene exposed workers must therefore be evaluated with caution.

Although the binding of a xenobiotic molecule to DNA may in principle be a genotoxic event. It should be kept in mind that "binding levels" in or below such orders of magnitude (7 adducts/10^{8} nucleotides) are well in the range of normally occurring molecular events (Ames and Gold, 1990).

3.3. CONCLUSION

Toxicokinetic studies reported here have shown species differences in the kinetics of styrene. This is confirmed using experimental data in PB-PK models comparing internal styrene oxide dosimetry (AUC_{SO}) in the blood of laboratory animals and man under various exposure conditions to styrene and styrene oxide. Such AUC_{SO} values have been used to examine the putative hazard of styrene for human beings. Comparison of predicted and measured AUC_{SO} values in animals with that in man exposed to an anticipated workplace concentration of 50ppm styrene shows AUC_{SO} values up to 400 times lower in man than in exposed animals in which no oncogenic effects were seen. In addition, the results of DNA binding studies showed that styrene metabolism in the rat or the mouse does not lead to the production of DNA binding intermediates. It is concluded that occupational or environmental exposure to styrene is unlikely to present any genotoxic or carcinogenic hazard to man.

APPENDIX

REVIEW OF THE TOXICOKINETICS, MACROMOLECULAR BINDING, CARCINOGENICITY AND MUTAGENICITY DATA FOR STYRENE AND STYRENE-7, 8-OXIDE

Appendix A	Toxicokinetics
Appendix B	DNA-Haemoglobin Binding
Appendix C	Carcinogenicity
Appendix D	Mutagenicity

APPENDIX A. TOXICOKINETICS

The occurrence of target organ toxicity is dependent on a host of factors including the movement of the parent compound and metabolites into and out of the target organ, as well as the rates of formation and degradation of the ultimate toxicant in the target organ. For Styrene, this is particularly complex since multiple isozymes activate styrene to styrene oxide (e.g., cytochrome p-450) and inactivate styrene oxide (e.g. glutathione S-transferase and epoxide hydrolase).

A.1. METABOLISM

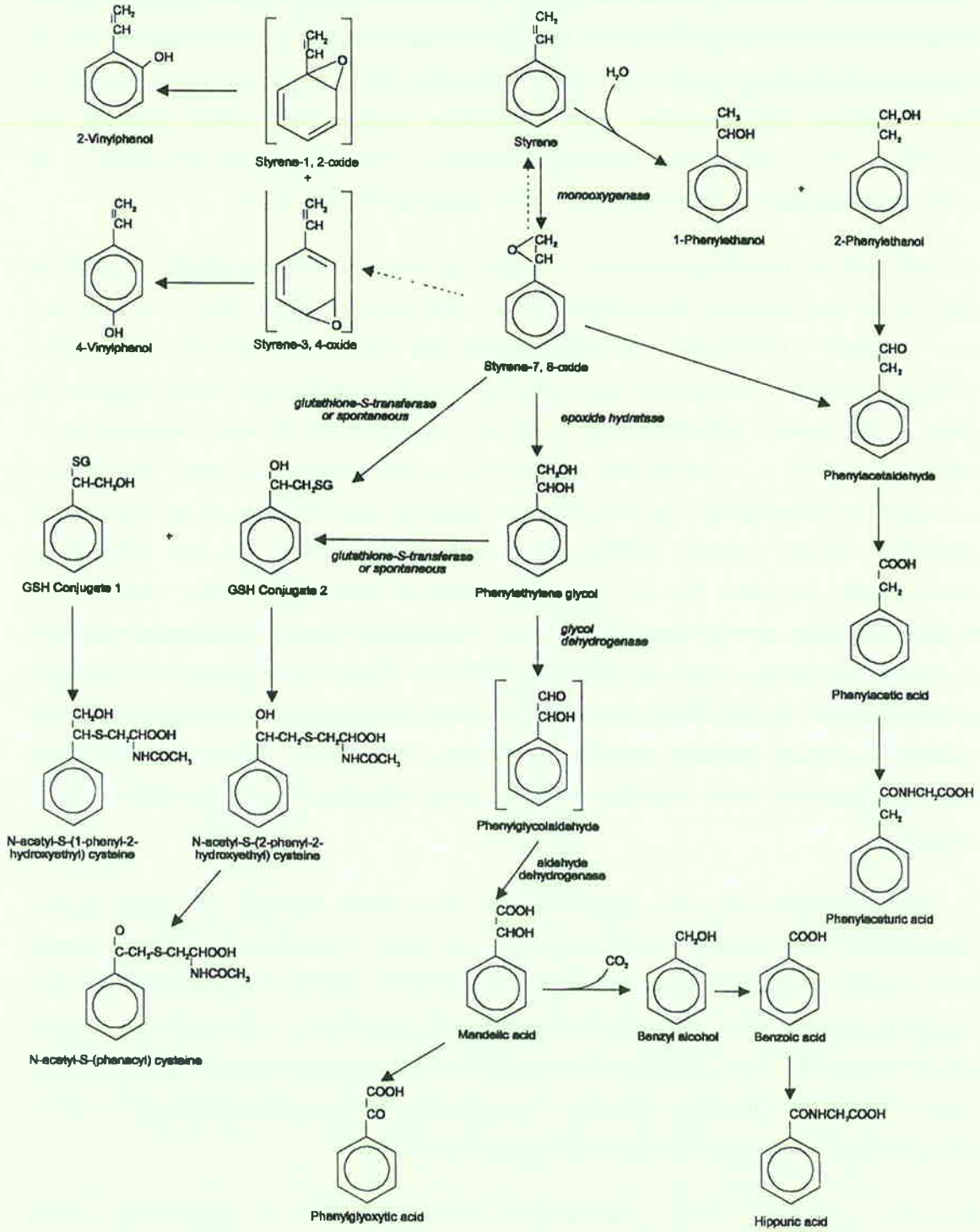
The metabolic fate of styrene is shown in Figure A-1. In both human beings and rodents, styrene is initially biotransformed to styrene-7, 8-oxide (styrene oxide) via microsomal cytochrome P-450 monooxygenases. Once formed, styrene oxide is either conjugated with glutathione via cytosolic enzymes or converted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

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

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

The difficulties and limitations of using the urinary metabolites as a biological indicator for styrene exposure have been addressed by Guillemin and Berode (1988). As a result of the many complexities involved, the styrene exposure estimates are highly uncertain in many of the published human studies, when based only on urinary metabolite measurements.

FIGURE A-1
METABOLIC FATE OF STYRENE



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

An additional minor metabolic pathway involves the production of vinylphenols, possibly via arene oxide intermediates. Bakke and Scheline (1970) reported that, in rats, 0.1% of an oral dose of styrene (100mg/kgbw in propylene glycol) was eliminated in urine as 4-vinylphenol. Conjugates of both 2-vinylphenol and 4-vinylphenol have been reported at low levels in the urine of rats injected intraperitoneally (dose not specified) with styrene (Hiratsuka *et al*, 1982). Pantarotto *et al* (1978) also reported that 4-vinylphenol was a minor metabolite in rats given an intraperitoneal injection of styrene, although again the dose of styrene was not specified. *In vitro* covalent binding studies with proteins of the rat liver endoplasmic reticulum also suggested that an arene oxide may by formed as a minor metabolite of styrene (Pantarotto and Blonda, 1984). In man, 4-vinylphenol has been detected in the urine of workers exposed to a mean styrene concentration of 130ppm; the 4-vinylphenol metabolite amounted to only about 0.3% of the amount of urinary mandelic acid, indicating that the ring oxidation is a minor metabolic pathway (Pfaffli *et al*, 1979; 1981). However, the possibility that 4-vinylphenol in urine may have been due to an impurity in the styrene has not been excluded.

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

Belvedere and Tonsi (1977) evaluated the kinetic behaviour of microsomal styrene monooxygenase and styrene oxide hydratase in male Sprague-Dawley rats, Swiss mice,

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

Tissue and species differences in the enzymes involved in styrene oxide metabolism have also been shown by Pacifici *et al* (1981). Using (7-^{3}H) styrene oxide as substrate, GSH S-transferase and styrene oxide hydratase activities were identified in the liver, lung and kidney of nine species, including man. In all species, the activities of both enzymes were higher in the liver than in the lung or kidney. The baboon had the highest hepatic styrene oxide hydratase activity (31nmol/mg/min) while the mouse had the lowest hepatic activity of this enzyme (1.9nmol/mg/min); the human styrene oxide hydratase activity (12.7nmol/mg/min) was intermediate between the mouse and baboon. Rodent species had higher GSH S-transferase activities than non-rodent species, with mouse liver having much higher activity (149nmol/mg/min) than in rats (87nmol/mg/min) or in man (25nmol/mg/min). The authors concluded that in rodents styrene oxide should be preferentially detoxified by conjugation with glutathione in all tissues, whereas in non-rodent species both conjugation and hydration should play a significant role. Since the *in vivo* importance of the GSH S-transferase pathway has not been demonstrated in all rodent species, e.g., the mouse, it is possible that the *in vitro* enzymatic activities are misleading and not representative of *in vivo* conditions. It

is also possible that the importance of the GSH S-transferase pathway is dose-dependent in certain rodent species such as the mouse.

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

In the isolated, perfused rat liver and rabbit lung preparations, conjugation with glutathione was a major metabolic pathway; nevertheless, significant amounts of diol were also formed in each instance. In the rat liver, 27-40% of the administered styrene oxide was excreted via the bile as a glutathione derivative {S-(1-phenyl-2-hydroxyethyl) glutathione}. No significant covalent binding of radiolabelled styrene oxide was detected in the livers used in these organ perfusion experiments.

The perinatal development of epoxide hydratase and GSH S-transferase was followed in foetal and neonatal guinea pigs, and the rates at which the enzymatic activities reached adult levels in various extrahepatic tissues differed from liver in both species. Moreover, the two enzymes developed at different rates in each organ, indicating that the relative importance of these two detoxification pathways may shift before and after birth.

Studies of the perinatal development of styrene monooxygenase and epoxide hydrolase in the rat liver showed that nuclear enzymes developed earlier than the corresponding microsomal activities (Romano *et al*, 1983). In both microsomes and nuclei, the development of styrene oxide hydratase was slightly slower than styrene monooxygenase; the capacity to detoxify the epoxide is poor before and early after birth, and thereafter increases with age. This observation suggests that newborn animals may be more sensitive than adults to styrene oxide and other epoxides. Further studies with rabbits indicated that there is a dissociation in the development of styrene monooxygenase and epoxide hydrolase activities in that species, in contrast to rats where both ontogenic and chemical induction studies suggest that these two enzymes are closely linked in their phenotypic expression (Romano *et al*, 1985).

The detoxification of styrene oxide by human liver GSH S-transferase has been studied by Pacifici *et al* (1987). GSH S-transferase activity in adult human liver is known to reside in several basic isozymes with isoelectric points between pH 8 and 10, and one near neutral form (GSTμ) with an isoelectric point between pH6 and 7. The GSTμ form is present in about one half of the population, and is known to be more active than the basic ones in the conjugation of glutathione with epoxides such as styrene oxide (Warholm *et al*, 1981). Individuals lacking the GSTμ isozyme may have a lower capacity to detoxify styrene oxide than those which have such an isozyme, although in general the GSH S-transferase pathway is believed to be of minor importance in man in comparison to the epoxide hydratase pathway.

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

A.2. EFFECTS OF STYRENE ON XENOBIOTIC METABOLISING ENZYMES.

Induction of the mixed function oxidase (MFO) system by treatment of rats with phenobarbital has been shown to increase the rate of metabolism of styrene. By contrast, the rate of styrene metabolism is decreased by administration of SKF-525A, an inhibitor of the MFO system, or by co-administration of other solvents requiring metabolism (Ohtsuji and Ikeda, 1971; Ikeda *et al*, 1972; Ikeda and Hirayama, 1978; Vainio and Zitting, 1978).

Comparison of the binding parameters for the interaction of styrene with non induced, phenobarbital-induced, and 3-methylcholanthrene-induced microsomes indicated that styrene is predominantly bound by cytochrome P-450 and not by cytochrome P-448 (Vainio and Zitting, 1978).

Parkki *et al* (1976) evaluated the effects of styrene and its metabolites, styrene oxide and styrene glycol, on xenobiotic metabolizing enzymes in rat liver. Intraperitoneal (ip) doses of

500mg/kgbw styrene in corn oil daily for three or six days was reported to double the activities of microsomal p-nitroanisole O-demethylase and epoxide hydratase (styrene oxide as substrate), whereas the activity of aryl hydrocarbon hydroxylase was practically unaffected. Non-statistically significant increases in cytochrome c reductase activity and cytochrome P-450 levels were noted only after six doses of 500mg/kgbw. Glucuronyl transferase activity was not affected by styrene if measured from native microsomes. However, when microsomes were pretreated with either digitoxin or trypsin, an approximate doubling of the glucuronyl transferase activity was noted for rats given six doses of 500mg/kgbw styrene. The glycine conjugation route was not affected by ip administration of 1,000mg/kgbw styrene for 3 days. A single dose of 375mg/kgbw styrene oxide resulted in a significant decrease in the activities of benz-(a)-pyrene hydroxylase and p-nitroanisole O-demethylase and in cytochrome P-450 content. Conversely, epoxide hydratase and NADPH cytochrome C reductase activities were not significantly altered by styrene oxide. Styrene glycol did not significantly alter the activities of the various xenobiotic metabolizing enzymes. The authors noted that styrene had differential effects on epoxide hydratase and cytochrome P-450, although epoxide hydratase is known to be located in the microsomal membrane intimately linked with cytochrome P-450 (Oesch, 1972) and suggested that the induction of cytochrome P-450 is not under that same control as that of epoxide hydratase. The enhancement of the activity of epoxide hydratase after styrene administration was thought to suggest that the rodent liver may be able to increase its capacity to metabolize styrene oxide in response to chronic styrene exposure.

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

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

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

The effects of styrene inhalation exposure on the xenobiotic metabolizing enzymes of male Wistar rat liver and kidney were also evaluated by Vainio *et al* (1979). Intermittent inhalation exposure of adult male rats to 300ppm styrene vapours, 6 hr/day, 5 days/week for up to 11 weeks enhanced the activities of ethoxycoumarin O-deethylase, epoxide hydratase, and glucuronyl transferase in the liver and kidneys. Cytochrome P-450 levels of hepatic microsomes were doubled after 2-weeks exposure and remained significantly higher than controls after 11 weeks. In kidneys, cytochrome P-450 levels were significantly elevated only after 11 weeks of exposure. Increased ethoxycoumarin O-deethylase activity was noted in both liver and kidney after only 2-weeks of exposure, whereas significant enhancement of glucuronyl transferase activity was not observed until after the 6th week of exposure. Epoxide hydratase activity was not determined after 2-weeks, but was found to be higher than for controls when measured after 4-, 8-, and 11-weeks of exposure (statistically significant only at 11-weeks). In contrast to the enhancements in hepatic enzymatic activities, further studies by the same group indicated that a single exposure to 500ppm styrene for 24hrs resulted in substantial decreases pulmonary cytochrome P-450 and in the activities of various pulmonary monoxygenase enzymes (Elovaara *et al*, 1990). These pulmonary enzymatic changes were associated with decreased pulmonary NPSH levels, as well as a 70-fold increase in urinary thioether excretion. The significance of these observations following a 24 hr exposure regimen is uncertain, since only slight decreases (mostly not statistically significant) were noted following a more relevant exposure regimen (500ppm, 5hrs/day, for 5 days). The authors concluded that the biochemical changes were unlikely to be caused by direct irritation, but substantiation of that conclusion was not provided.

Das *et al* (1981) reported a dose-dependent increase in hepatic aminopyrene- and ethylmorphine-N-demethylase activities as well as arylhydrocarbon hydroxylase and aniline

hydroxylase activities in rats given high oral doses of styrene (450 or 900mg/kgbw for 7 days). These investigators also reported that GSH S-transferase activities were lower in rats given 450 or 900mg/kgbw styrene than in control animals.

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

Mendrala *et al* (1992) recently evaluated the *in vitro* activities of enzymes involved in the formation and degradation of styrene oxide in liver and lung tissues from rats and mice, as well as in human liver tissue. Based on the Vmax for styrene epoxidase activity and the relative liver and body size, mice were found to have the greatest capacity and human beings the lowest capacity to form styrene oxide from styrene. Human epoxide hydratase was found to have a greater affinity (i.e., lower Km) for styrene oxide than epoxide hydratase from rats and mice, indicating that human liver is more effective than rodent liver in hydrolysing low levels of styrene oxide formed from styrene. Prior exposure of rats (1,000ppm, 6hrs/day for 4 days) or mice (600ppm, 6hrs/day for days) to styrene had no apparent effect on styrene epoxidase activity or GSH S-transferase activity in either liver or lungs of rats or mice. However, there was a 1.6-fold increase in the activity of hepatic styrene oxide hydratase in rats pre-exposed to styrene; this was consistent with the observation previously reported by Parkki *et al* (1976). Prior exposure to styrene had no apparent effect on Km or Vmax of hepatic styrene epoxidase in either rats or mice. Assuming that repeated-dose styrene toxicity is due primarily to styrene oxide, these *in vitro* studies confirm the observation that mice are more sensitive to styrene than rats, and suggest that the rodents would be more sensitive than humans.

A.3. ELIMINATION

Human studies have shown that 90-97% of absorbed styrene is eliminated as urinary metabolites (Guillemin and Bauer, 1978; Ramsey and Young, 1978), while only a small fraction is eliminated as unchanged styrene in urine or expired air (Stewart *et al*, 1968; Imbriani *et al*, 1985). The postexposure urinary elimination of mandelic acid is apparently biphasic, with the half-time for the first phase (<20 hours after exposure) being about 4-9 hours, and the second phase (>20 hours after exposure) about 17-26 hours (Engstrom *et al*, 1976, 1978a; Guillemin and Bauer, 1979). The urinary elimination of phenylglyoxylic acid seems also to be biphasic, with the half time for the first phase (<50 hours after exposure) being about 10 hours, and the second phase (50-200 hours after exposure) about 26 hours (Caperos *et al*, 1979; Guillemin and Bauer, 1979). Studies with human volunteers indicate that the half-life of styrene in adipose tissue is about 2-4 days (Engstrom *et al*, 1978b).

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

A.4. TOXICOKINETICS

The toxicokinetics of styrene in rats and man were described by Ramsey and Young (1978). Human volunteers were exposed to 80ppm styrene for 6 hours, and rats were exposed to concentrations ranging from 80 to 1,200ppm for 6 hours. For both rats and man exposed to 80ppm, the clearance of styrene from blood could be described with a two-compartment linear pharmacokinetic model. However, when rats were exposed to higher concentrations between 200 and 600ppm, the clearance process from blood became saturated. For example, the maximum blood concentration of styrene in rats increased almost 80-fold when the exposure concentration was increased 15-fold from 80ppm to 1,200ppm. The results suggest that the rat may be a good animal model for understanding the pharmacokinetics and toxicity of styrene, at least at concentrations up to 80ppm. Anderson and Ramsey

(1983) developed a physiologically-based pharmacokinetic (PB-PK) model which accurately described the nonlinear behaviour of styrene in rats exposed to high concentrations; this model was also able to represent accurately the data from human beings exposed to 80ppm styrene. Expanding on this work, Ramsey and Anderson (1984) simulated routes other than inhalation, and applied the model to interspecies comparisons. The model predictions indicated that styrene metabolism is saturated at styrene exposure concentrations greater than 200ppm in rats, mice and man. Additional studies by Anderson *et al* (1984) focused on the pharmacokinetics in rats under conditions in which the metabolism of styrene was either induced or inhibited. Fischer 344 rats were exposed to styrene concentrations ranging from 100 to 2,000ppm. Subgroups of rats were pretreated with pyrazole (an inhibitor of styrene metabolism), phenobarbital (an inducer), or styrene (1,000ppm, 6hrs/day, 4 days). Pretreatment with pyrazole essentially abolished styrene metabolism, while phenobarbital increased Vmax about 6-fold, and styrene increased Vmax by a factor of 2.

The PB-PK model is currently being expanded to include consideration of styrene-oxide as an intermediate metabolite of styrene (Filser and Nolan, in press). The levels of styrene oxide in blood are expected to be low as a result of the rapid rate at which it is hydrolysed and conjugated. However, Lof *et al* (1984) reported measurements of styrene oxide in several tissues of mice intraperitoneally dosed with radiolabelled styrene. Styrene oxide was also reported at levels ranging from 0.02µg/ml to 0.05µg/ml in venous blood samples from several human subjects exposed to styrene (Wigaeus *et al*, 1983 and 1984; Lof *et al*, 1986a,b). However, the *in vivo* measurements of styrene oxide in these studies involved analytical methods in which styrene oxide was detected only indirectly via acid hydrolysis to styrene glycol.

Due to the potential for analytical errors with the indirect methods, extremely sensitive direct analytical methods have recently been developed (Kessler *et al*, 1990; Langvardt and Nolan, 1991). These direct methods were in close agreement in showing that the *in vitro* half-life of styrene oxide in rat whole blood was in the range of 24-26 minutes at 37°C, at an initial blood concentration of approximately 10µg/g. The unexpectedly long half-life of styrene oxide in whole blood *in vitro* indicates that styrene oxide is not as reactive as was hitherto assumed. Kessler *et al* (1990) found that at steady state, the styrene oxide blood concentrations ranged from 0.008µg/ml to approximately 0.45µg/ml in male Sprague-Dawley rats exposed to styrene air concentrations of 20ppm to 800ppm, respectively. Near maximum styrene oxide steady state blood levels were attained in rats exposed to 260ppm styrene, and exposure to higher styrene concentrations resulted in very little increase in styrene oxide blood levels.

Using the Langvardt and Nolan (1991) method Nolan *et al* (1991) found that a single styrene oxide oral dose of 550mg/kgbw resulted in peak blood levels of 12.9µg/g in rats. During the first 10 hours after rats were given a single 500mg/kgbw oral dose of styrene, the styrene oxide blood levels were relatively constant between 0.093 and 0.172µg/g, confirming that the metabolism of styrene was saturated. Thus, the blood styrene oxide concentrations in rats in this study represent the highest concentrations of styrene oxide that can occur in rats exposed orally to styrene. While the peak styrene oxide blood concentrations were much lower (<2%) after oral administration of 500mg/kgbw of styrene than after 550mg/kgbw of styrene oxide, the area under the blood styrene oxide concentration-time curve (AUC) for the styrene dose was almost half of the AUC for the styrene oxide dose.

Mendrala *et al* (1992) also measured styrene and styrene oxide concentrations in blood of naive and pre-exposed rats given a single oral dose of 500mg/kgbw styrene. Styrene blood levels were similar in the naive and previously exposed animals. Blood styrene oxide concentrations, on the other hand, seemed to be somewhat higher in naive rats than in those previously exposed to styrene, although the observed differences were not statistically significant. Nevertheless, the suggestion of lower blood styrene oxide levels in previously exposed rats is consistent with the apparent increase in hepatic styrene oxide hydratase activity in pre-exposed animals (see section A.1).

In summary, styrene is rapidly and extensively metabolized. Mandelic acid and phenylglyoxylic acid are the two major urinary metabolites in humans. The metabolism of styrene proceeds via styrene-7,8-oxide (styrene oxide), but recent studies have shown that there are pronounced species differences in the internal styrene oxide dosimetry following exposure to styrene. Rodents have a greater capacity than humans to form styrene oxide from styrene, while humans are more effective than rodents in detoxifying low levels of styrene oxide. The low levels of styrene oxide that could occur in humans under foreseeable styrene exposure conditions are very unlikely to pose a human health hazard.

APPENDIX B. MACROMOLECULAR BINDING

B.1. HAEMOGLOBIN BINDING

The concept of using haemoglobin adducts as a measure of internal dose has been reviewed by a number of authors (Ehrenberg and Osterman-Golkar, 1980; Farmer *et al*, 1987; ECETOC, 1989). A variety of amino acid residues present in haemoglobin (including cysteine, histidine, N-terminal valine and C-terminal carboxylic acid) have been shown to be modified by a range of chemicals. Analytical methods used to measure such modifications include gas chromatography (GC) with mass spectrometry (MS) or electron capture detection (EDC). Using such approaches attempts have been made to develop styrene haemoglobin binding methodology for monitoring styrene exposure.

A number of *in vitro* studies have demonstrated the ability of styrene oxide to react with a number of different polyamino acids (Hemminki, 1983, 1986a,b) with an affinity : cysteine > histidine > lysine > serine; similar experiments with erythrocytes also showed the predominance of cysteine derivatives. A report from Kaur *et al* (1989), which also examined *in vitro* binding of styrene oxide with amino acids or erythrocytes, reported modification of histidine as well as cysteine.

Haemoglobin binding in experimental animals treated with styrene or styrene oxide has been reported by a number of investigators including Nordqvist *et al* (1985) and Sepai *et al* (1992). In the first of these studies the presence of a reaction product, bound to N-terminal amino acid valine of haemoglobin was found in mice treated by intraperitoneal (i.p.) injection with radiolabelled styrene (dose 0.12 to 4.9mmole styrene/kg body weight) or styrene oxide (dose 0.063 to 1.1mmole styrene oxide/kg body weight). Sepai *et al* (1992) reported the successful use of GC-MS to determine styrene oxide carboxylic acid ester in haemoglobin of rats treated with large doses of the oxide.

A common effect noted in the animal studies was that while a linear correlation between treatment and binding existed at low exposure levels when dose levels were increased (for styrene oxide approx >50mg/kgbw) there was a marked deviation from linearity characterised by an disproportionate increased in haemoglobin binding. The results are consistent with the toxicokinetic data which has demonstrated that following high applied doses of styrene or styrene oxide the metabolising system becomes overwhelmed resulting in high systemic levels of the oxide characterised by the higher haemoglobin binding values. Quantitative differences in haemoglobin binding between species (i.e. at comparable exposures to styrene or styrene oxide protein binding is 3-fold higher in the mouse as compared with the rat -

such differences becoming more pronounced with increasing dose levels) also equates to the pharmacokinetic variation between the species (see 2.2.1).

In a preliminary study analysing globin samples from a population of 14 styrene exposed boat builders Sepai *et al* (1992) found no evidence of adduct formation at a detection limit of 15pmole/g globin - unfortunately no information is provided on levels of exposure. In contrast Brenner *et al* (1991) reported the presence of styrene haemoglobin adducts in a population of 14 fibreglass-reinforced plastic boatbuilders (styrene exposure range 0.6 to 44ppm) as well as in 8 control individuals. The data showed that the mean level of haemoglobin binding was higher in the boatbuilders, though not significantly so, as compared to the control group. The increased was, in the main, due to a high level measured in a single worker. Apart from the low numbers used in the study another serious confounder can be found in the smoking status of the groups, i.e. approx 50% smokers in the occupational group as compared with 0% in the controls. As cigarette smoke is known to contain quite high levels of styrene the slightly higher adduct levels in the boatbuilders could be equally associated with cigarette smoking as with occupational exposure to styrene.

B.2. DNA BINDING

Methods which have been used to examine the potential for styrene or styrene oxide to bind with DNA include covalent binding studies with radiolabelled chemicals and (^{32}P) postlabelling. A brief description of such methods can be found in ECETOC (1989).

Styrene oxide has been found to form covalently bound adducts with nucleophilic sites in isolated DNA *in vitro*. In the studies by Savela *et al* (1986) and Vodicka and Hemminki (1988) the major reaction site was reported as being the N^7 position on guanine although N^2 and O^6 alkylation products were also detected. More products were formed in single strand DNA as compared with double stranded DNA. In an investigation using (^{32}P) postlabelling Pongracz *et al* (1992) reported the N^2-guanosine derivatives were the major products of the reaction of isolated DNA and styrene oxide.

The extent of covalent binding to guanine N^7 in DNA isolated from mice treated (i.p. injection) with radiolabelled styrene or styrene oxide has been investigated by Nordqvist *et al* (1985). The authors reported that DNA binding, in mice receiving the same dose of either styrene or styrene oxide (i.e. injected amount of 1mmol/kg body weight), was 17(±5) or 8(±2)nmole guanine N^7 adduct/kg body weight respectively. These results, reporting quite high levels of binding and effects, are in contrast to the data of Lutz (1992) and Cantoreggi and Lutz (1992 and in press see 3.2.2) which showed no binding at the detection level or only minute effects. Cantorreggi and Lutz, (1992) point out that Nordqvist *et al* (1985) apparently did not

purify the DNA samples thus it is possible that the DNA samples had not been freed of protein-bound or non-covalently bound radiolabelled material.

The (^{32}P) postlabelling technique was used by Liu *et al* (1988) to detect styrene oxide adducts in DNA isolated from lymphocytes. Two workers were examined; the first, exposed to a level of 96ppm styrene, showed evidence of adduct formation while in the second, unexposed worker, no DNA adducts were detected. A second report from the same laboratory (Bodell *et al*, 1990) also reported the presence of adducts, detected by ^{32}P postlabelling, in DNA isolated from lymphocytes obtained from a worker occupationally exposed to styrene; average exposure on day of blood collection was 47ppm. It is not possible to determine if both papers refer to the same or different individuals. In the EEC sponsored study (STEP, 1988) lymphocytes were also collected from workers with exposure to styrene. DNA isolated from six of the lymphocyte samples (selected on the basis of high levels of urinary mandelic acid -levels not provided) was examined by the ^{32}P-postlabelling technique. No styrene related adducts were detected in any of the samples.

As described previously there are, currently, many problems associated with both the analytical methodology used in ^{32}P-postlabelling as well as with interpretation of the results. For example the presence of endogenous adducts (Phillips *et al*, 1986; Reddy *et al*, 1990), smoking (Jahnke *et al*, 1990; Savela and Hemminki, 1991) diet related adducts (Rothman *et al*, 1990) and/or inter-individual variation can all influence the nature of the results. Once such problems are resolved the ^{32}P-postlabelling technique could provide useful information in the area of biomonitoring and assessment of possible health effects.

APPENDIX C. CHRONIC TOXICITY/CARCINOGENICITY

C.1. STYRENE EPIDEMIOLOGY STUDIES

The epidemiology data on styrene is quite substantial, and has been reviewed recently by Bond *et al* (1991). The available data includes eight cohort mortality studies which have focused on workers employed in either the reinforced plastics manufacturing industry or in styrene monomer, polymer and styrene-butadiene rubber manufacturing operations. Collectively, these eight studies have included nearly 50,000 employees during the time period between 1940 and 1986.

The results from the eight cohort mortality studies are summarized in Table C-1. The two most recent studies, those by Coggon *et al* (1987) and by Matanoski and Schwartz (1987), demonstrated deficits for almost all subtypes of lymphatic and haematopoietic cancer, including some that were markedly diminished. The study by Okun *et al* (1985) found no deaths from lymphatic and haematopoietic cancer in a large but relatively young cohort of reinforced plastics boatbuilders with limited follow-up. Wong (1990) reported 5 deaths from leukemia, compared with 4.76 expected, and 9 deaths from the combined category of lymphatic and haematopoietic cancer versus 12.3 expected among a large group of nearly 16,000 workers in the reinforced plastics industry. Nicholson *et al* (1978) found one leukemia and one lymphoma and concluded that, although their data were not definitive, the environmental risk from styrene was "not extraordinary".

Three of the eight studies reported excess deaths and/or incidence from cancer of the lymphatic and haematopoietic systems. Meinhardt *et al* (1982) reported an elevated, but not statistically significant, leukemia mortality among employees from two styrene butadiene rubber (SBR) facilities which they interpreted as suggestive of an association. Five of the six cases were of the myelogenous type. Ott *et al* (1980) found a statistically significant excess incidence of lymphatic leukemia among workers involved in colorant blending, roll compounding or extrusion of plastics. This operation was characterized by complex, multiple chemical exposures, and the possibility that the tumours were induced by styrene was diminished by the absence of any leukemia cases among the subgroup of workers with the highest styrene exposures. A statistically significant excess in lymphoma mortality (believed to be non-Hodgkin's lymphoma) was reported among workers producing styrene monomer and polymers (Hodgson and Jones, 1985).

All three of the foregoing studies that reported excess cancer of the lymphatic and haematopoietic systems were conducted on groups of workers with mixed chemical

TABLE C-1

SUMMARY OF FINDINGS FROM EIGHTS STUDIES OF STYRENE WORKERS

Reference	All leukemia		Malignant Lymphoma						All cancer of lymphatic and hematopietic	
			HD		NHL		All			
	O	E	O	E	O	E	O	E	O	E
Meinhardt *et al*, 1982	6.0	3.5	1.0	1.4	4.0	2.2	5.0	3.8	11.0	8.3
Ott *et al*, 1980	6.0	3.4	NR	NR	NR	NR	7.0	5.3	13.0	8.7
Hodgson and Jones, 1985	0.0	0.3	NR	NR	NR	NR	3.0	0.6	3.0	0.9
Coggon *et al*, 1987	3.0	6.0	1.0	2.4	1.0	4.2	2.0	6.6	6.0	14.9
Matanoski and Schwartz, 1987	17.0	18.7	8.0	6.7	5.0	10.2	13.0	16.9	40.0	47.1
Okun *et al*, 1985	0.0	1.7	NR	NR	NR	NR	0.0	2.1	0.0	4.2
Wong, 1991	5.0	4.8	3.0	2.4	0.0	2.6	3.0	5.0	9.0	12.3
Nicholson *et al*, 1978	1.0	0.8	NR	NR	NR	NR	1.0	1.3	2.0	2.0

NR Not reported
O Observed number of deaths
E Expected number of deaths

exposures including benzene, a known human leukemogen. Each study pointed to a different tumour type, making it unlikely that a single environmental factor was involved. Furthermore, none the studies found evidence for a dose-response relationship, although attempts were hampered somewhat by the lack of precise exposure measurements.

The combined weight of the evidence is the ultimate basis for evaluating the potential of a substance to cause cancer in human beings. The data from the eight studies were therefore pooled by Bond *et al* (1991) in order to gain additional perspective. The pooled results are presented in Table C-2. In the major subcategories, lymphoma and leukemia, as well as for the entire category of lymphatic and haematopoietic cancer, only deficits in mortality are seen.

TABLE C-2
POOLED RESULTS FROM EIGHT STUDIES OF STYRENE WORKERS

Cancer Type	Obs	Exp	SMR	95% CL
Total Lymphatic and Hematopoietic Cancer	84.0	98.4	85.0	68-10
All Leukemia	38.0	39.1	97.0	69-13
Malignant Lymphoma				
All	34.0	41.5	82.0	51-10
Hodgkin's disease	13.0	12.9	101.0	54-17
Non-Hodgkin's	10.0	19.5	51.0	25-9

Obs Observed number of deaths
Exp Expected number of deaths
SMR Standardised mortality ratio = (Obs/Exp) x 100
95% CL 95% confidence interval about the SMR

In summary, the pooled weight of the evidence from the substantial epidemiologic data on styrene shows no indication of carcinogenicity related to styrene exposure.

C.2. STYRENE LONG-TERM ANIMAL STUDIES

A total of nine long-term animal studies have been conducted on styrene, and two additional long-term studies have been conducted on a mixture of styrene and β-nitrostyrene. These studies are briefly summarized in Tables C-3 and C-4 for rats and mice, respectively. Although most of the long-term animal studies on styrene have specific deficiencies or limitations which preclude definitive conclusions, the available data are nevertheless substantial and quite complex.

TABLE C-4
SUMMARY OF STYRENE CARCINOGENICITY STUDIES WITH MICE

Strain	Route	No/ group	Exposure level	Schedule	Duration	Tumor site/type	Comments	Reference
O20	Gavage	45 ♂ 39 ♀	1350 mg/kg	weekly	16 weeks. observation for lifetime	increase in frequency and earlier appearance of spontaneously occuring lung tumor	treatment suspended after 16 weeks due to toxicity. extermely high mortality.	Ponomarkov and Tomatis, 1978
C57BL	Gavage	27 ♂ 27 ♀	300 mg/kg	weekly	120 weeks.	non-statistically significant increase in liver tumors in males		Ponomarkov and Tomatis, 1978
B6C3F1	Gavage	50 ♂ 50 ♀	150, 300 mg/kg	5d/wk	78 weeks. observation until week 91	significant increase in lung tumors in high dose males compared to current but not historical controls. non-statistically significant increase in hepatocellular ademona in females.	dose-related increase in mortality in males. dose-related decrease in weight gain in females	NCI, 1979
B6C3F1[a]	Gavage	50 ♂ 50 ♀	203, 407 mg/kg	3d/wk	78 weeks. observation until week 92	non-dose related increase in lung tumors of males in low dose group only		NCI, 1978

a Test material was 30% β-nitrostyrene and 70% styrene

Ponomarkov and Tomatis (1978) conducted oral gavage bioassays on styrene, initiated *in utero* on two strains of mice (020 and C57BL), as well as on the BDIV rat. The design of these studies was unusual in that pregnant animals were given oral doses at day 17 of gestation, and the progeny were then given weekly doses. In the O20 mouse study, both the dams and progeny (45 males and 39 females) were administered doses of 1,350mg/kgbw. Treatment of progeny was stopped after 16 weeks because of mortality. There were two control groups: one control group (20 males and 22 females) was given weekly doses of 0.1ml of olive oil for life and the other control group (54 males and 47

TABLE C-3
SUMMARY OF STYRENE CARCINOGENICITY STUDIES WITH RATS

Strain	Route	No/ group	Exposure level	Schedule	Duration	Tumor site/type	Comments	Reference
BDIV	Gavage	73 ♂ 71 ♀	500 mg/kg	Weekly	120 weeks	no significant increase		Ponomarkov and Tomatis, 1978
Fischer	Gavage	50 ♂ 50 ♀	500, 1000, 2000 mg/kg	5/wk	103 weeks 1 wk obs (low dose) 78 weeks 27 weeks observation (high doses)	no significant increase	high mortality in top dose	NCI, 1979
Sprague-Dawley	Gavage	40 ♂ 40 ♀	50, 250mg/kg	4-5d/wk	52 weeks lifetime observation	no significant increase	increased mortality in top dose group	Conte et al, 1988
Sprague-Dawley	Drinking water	50 ♂ 70 ♀	125, 250ppm	ad lib	2 years	no significant increase	no increased mortality decreased H_2O cun-sumption decreased terminal body weight in high dose females	Belles et al, 1985
Sprague-Dawley	Inhal-ation	30 ♂ 30 ♀	25, 50, 100, 200 ,300 ppm	4hr/day, 5day/wk	52 weeks lifetime observation	non doses related increase in mammary tumors in females		Conte et al, 1988
Sprague-Dawley	Inhal-ation	40 ♂ 40 ♀	0, 25, 50, 100, 200, 300ppm	4hr/day, 5day/wk	52 weeks	no increase in brain tumors	only brain tissue examined for tumors	Maltoni et al, 1982
Sprague-Dawley	Inhal-ation	85 ♂ 85 ♀	600, 1000 pmm[a]	6hr/day, 5day/wk	♂-18.3 mn. ♀-20.7 mn. observation until 24 months	non-dose related increase in adenocar-cinomas of mammary gland in females; non-dose related, non-stat. signif. increase in combined leukemias and lymphomas in both sexes.	murine pneumonia in control and high dose males. decreased body weight in top dose groups. decreased body weights in 600ppm group for first 263days in males	Jersey et al, 1978
Fischer[b]	Gavage	50 ♂ 50 ♀	350, 700 ♂ 175, 350 ♀	3 d/wk	79 weeks observation until wk 108	no significant increase		NCI, 1978

a 1200ppm for first two months
b Test material was 30% β-nitrostyrene and 70% styrene

females) was untreated. The mean survival time of the male and female O20 mice given styrene was significantly less than that of the controls. A statistically significant increase in lung tumours was reported in male and female mice relative to the olive-oil control group, as well as for female mice versus the untreated control group. The dose of styrene given clearly exceeded the maximum tolerated dose as evidenced by the increased mortality in the weanlings and early mortality in the progeny of the styrene-treated mice. Interpretation of

these data is further complicated by the small number of animals involved in the study, and by the absence of data on litters as well as on the background incidence of pulmonary tumours in this strain of mice.

In the study with C57BL mice, the pregnant mice were given an oral dose of 300mg/kgbw of styrene on day 17 of gestation and the progeny (27 males and 27 females) were given weekly oral doses of 300mg/kgbw for life. Two control groups were used; these consisted of 13 female and 12 male progeny dosed with vehicle alone, and 51 male and 49 female untreated animals. The incidence of liver tumours observed in the treated mice, vehicle-treated controls, and untreated controls were 3 of 24 (12.5%), 1 of 12 (8.3%) and 1 of 47 (2.1%) mice, respectively. The difference between the incidence of liver tumours in the treated male mice (3 of 24) and the pooled control mice (2 of 59) was not statistically significant.

Pregnant BDIV rats were given an oral dose of 1,350mg/kgbw of styrene and the progeny (73 males and 71 females) were given weekly oral doses of 500mg/kgbw of styrene. Control group animals (36 males and 39 females) were given vehicle alone. There was no indication of an oncogenic response related to styrene exposure in any organ or tissue.

Jersey *et al* (1978) conducted a study in which male and female Sprague-Dawley rats were exposed to 0, 600ppm or 1,000ppm for 6hrs/day, 5 days/week, for 2 years. The study was initiated using a high exposure concentration of 1,200ppm, but early mortality in the males necessitated lowering the exposure concentration to 1,000ppm after 2 months. The study results were complicated by an outbreak of murine pneumonia unrelated to the styrene exposures which had a significant effect on all groups of animals, particularly on control and 1,000ppm males. A statistically significant increase in the incidence of mammary gland adenocarcinomas was observed in female rats in the low (600ppm) exposure group. The incidence of these tumours were: 1/85 (1.18%), 7/85 (8.23%) and 0/75 (0%) in female rats at 0, 600 and 1,000ppm, respectively. Hence, there was no dose-response relationship since the incidence of mammary gland adenocarcinomas in the high exposure group females was not different from controls. Moreover, the incidence of mammary gland tumours in the 600ppm group females was within the range of normal for historical control groups (range of 0 to 9.3%). The incidence of the combined diagnoses of leukemia plus lymphosarcoma (leukemia-lymphosarcoma) were higher in both exposure groups of female rats as well as in the 600ppm exposure group males than was observed in the controls. However, the differences were not statistically significant and, for females, there was no dose-response relationship since the incidence of leukemia-lymphosarcoma were the same, 6 of 85 (7.06%) in both the high and low exposure groups. For males, the incidence of leukemia-

lymphosarcoma was actually lower in the 1,000ppm exposure group 1 of 84 (1.19%) than in the 600ppm group, 5 of 86 (5.81%). Interpretation of the results of this study is seriously confounded by the excessive mortality and intercurrent infections.

Conti *et al* (1988) reported results of a non-peer reviewed study that was conducted between 1974 and 1977, in which styrene was administered to Sprague-Dawley rats by four different routes:

- intraperitoneal injection: 4 injections, 50mg per injection, two-month intervals between injections, administered to 40 male and 40 female rats:
- subcutaneous injection: a single injection of 50mg to 40 male and 40 female rats:
- inhalation: groups of 30 male and 30 female rats were exposed to 25, 50, 100, 200 or 300ppm styrene for 4 hour day, 5 day/week for 1 year, sixty rats of each sex served as controls:
- ingestion: groups of 40 male and 40 female rats were given 50 or 250mg styrene/kgbw, in olive oil, 4-5 day/week for 1 year.

The authors reported that there were no indications of an oncogenic response in any of the studies except in the inhalation study, where increases in the incidence of total benign plus malignant mammary tumours (TBMT) and of malignant mammary tumours (MT) was noted in all exposed female groups. The incidence of MT were 20, 13, 30, 40 and 30% in the 25, 50, 100, 200 and 300ppm exposure groups, respectively, while the control group incidence was 10%. For TBMT, the control incidence was 56.7%, with incidence levels of 80, 70, 70.7, 80 and 83.3% for the corresponding exposure groups. Hence, there was no clear dose-response relationship, since the incidence of both MT and TBMT were essentially the same in each of the exposure groups. The results of this study are difficult to evaluate since no mortality data was included, and no information was provided about the incidence of mammary tumours in historical control groups of animals. Furthermore, statistical analyses were not conducted, and the tumour data was reported only as percentages.

In a 1982 publication which may or may not relate to the Conti *et al* (1988) report, Maltoni *et al* (1982) indicated that there was no increase in brain tumours in male or female rats given daily oral doses of 50 or 250mg/kgbw styrene in olive oil, 4-5 days per week, for 52 weeks.

Beliles *et al* (1985) conducted a study in which styrene was administered to Sprague-Dawley rats in their drinking water at levels of 125 and 250ppm for two years. Using the highest dose the concentration approached the saturation level in water (approximately 300ppm),

and therefore represents the maximum possible dose in drinking water. During the two year exposure period, males and females were randomly selected for breeding the F1 generation, and then returned to the chronic bioassay. The F1 generation was also exposed to styrene in drinking water and mated at around 110 days of age to derive an F2 generation. Subsequently, an F3 generation was also derived. The calculated daily dose of styrene was stated as being 7.7 and 14mg/kgbw/day for male rats and 12 and 21mg/kgbw/day for females. The authors of the study concluded that there were no gross or histologic dose-related changes nor evidence of carcinogenicity, and no deleterious dose-related effects or decrements in reproductive function through three generations. Except for a statistically significant reduction in water consumption for both males and females of both dose groups (related to unpalatability), no treatment-related changes were reported for animals in the chronic study.

The National Cancer Institute (NCI) (1979) conducted a study using Fischer 344 rats and B6C3F1 mice. In the study with Fischer 344 rats (50/sex/dose group), oral doses of 0, 1,000, or 2,000mg/kgbw of styrene in corn oil were given by gavage 5 day/week for 78 weeks. Dosing was stopped after 78 weeks and the animals were observed for another 27 weeks. Because of the high incidence of mortality in rats given 2,000mg/kgbw, additional animals were added to the study and given oral doses of 0 or 500mg/kgbw of styrene in corn oil, 5 day/week, for 103 weeks. The 2,000mg/kgbw dose level exceeded the maximum tolerated dose as indicated by the excessive styrene-related mortality, and survival was inadequate to evaluate the risk from late developing tumours. However, there were no treatment-related increases in any tumour type in rats given either 500 or 1,000mg/kgbw dose level. In the study with B6C3F1 mice (50/sex/dose group), oral doses of 0, 150 and 300mg/kgbw of styrene in corn oil were administered by gavage 5 day/week for 78 weeks. Treatment was stopped after 78 weeks because of the high mortality in the mice given the 300mg/kgbw dose level, and the animals were allowed to live for an additional 13-week observation period. The only statistically significant increase in tumours observed in this study was an increase in combined alveolar/bronchiolar carcinomas and adenomas in male mice. The observed incidence of these tumours were 0 of 20 (0%) for controls, 6 of 44 (14%) for the low dose, and 9 of 43 (21%) for the high dose. The authors of the NCI report specifically noted the following points :

- the B6C3F1 mice have a high incidence of spontaneous pulmonary tumours,
- the incidence of pulmonary tumours in the control males (0%) was unusually low, and

- the incidence of pulmonary tumours in the high-dose male mice did not differ from the historical incidence of these tumours in untreated control mice, 32 of 271 (12%) maintained at the same laboratory.

The data on vehicle treated mice was considered to be insufficient for meaningful use as a historical control value. The authors concluded that, under the conditions of this bioassay, there was no convincing evidence of carcinogenicity in either male or female Fischer 344 rats or B6C3F1 mice.

A carcinogenicity study in rats and mice on a mixture of 30% β-nitrostyrene and 70% styrene was also reported by NCI (1978). Fischer 344 rats (50/sex/dose group) were orally administered styrene at dosages calculated to be 700 or 350mg/kgbw in males and 350 or 175mg/kgbw in females. B6C3F1 male and female mice (50/sex/dose group) were treated with 407 or 203mg/kgbw, respectively. Dosing was for 3 day/week for 79 weeks with further observation for 29 weeks (rat) or 14 weeks (mouse). No treatment-related increase in any tumour type was noted.

Quast *et al* (1979) conducted a long-term chronic toxicity study in which styrene was administered daily to Beagle dogs by oral intubation at dose levels of 200, 400 or 600mg/kgbw/day (in peanut oil) for up to 561 days. The only treatment-related manifestation of potential toxicity was associated with the development of an increased incidence of Heinz bodies and associated changes in red blood cells. A dose-related increase in the incidence of these intraerythrocytic inclusions bodies was detected in males and females from the 400 and 600mg/kg/day dose groups, and sporadically in females in the low dose group. Upon cessation of treatment, the erythrocytes containing Heinz bodies were rapidly removed from circulation, and all associated red blood cell parameters subsequently returned to normal. The Heinz body formation was not associated with increased numbers of reticulocytes, and light microscopic examinations revealed no evidence of bone marrow hyperplasia. In addition, serum bilirubin, urine bilirubin and urine urobilinogen levels were normal, thus indicating the red blood cell turnover rates were not unusually high. The significance of the Heinz body formation in this study is uncertain with regard to human risk estimations, since analogous changes have not been found in other species.

In summary, the available long-term animal studies provide no clear evidence of a carcinogenic response related to styrene exposure. This conclusion is supported by a weight-of-the-evidence approach based on the fact that several species showed no oncogenic response, together with the fact that there was no consistent target organ or effect (i.e., no organotropism) in the various studies that have been conducted to date.

A new long-term rat inhalation bioassay is currently in progress which will provide important additional perspective about the carcinogenic potential of styrene (SIRC, 1991).

C.3. STYRENE OXIDE LONG-TERM ANIMAL STUDIES

There have been a total of seven long-term animal studies on styrene oxide (Tables C-5 and C-6) five of those studies involved administration of the material at high doses by gavage, while in the other two studies it was applied repeatedly to the skin. Maltoni *et al* (1979) and Conti *et al* (1988) administered styrene oxide to Sprague-Dawley rats by gavage at dose levels of 50 or 250mg/kgbw/day in olive oil, 4-5 days/week, for 52 weeks, and the surviving animals were then held until spontaneous death for up to 52 additional weeks. A dose-related increase in incidence of forestomach neoplasias, papillomas and precursor lesions (acanthosis and dysplasia) was observed in the styrene treated groups. There was no indication of an oncogenic response in any other organ or tissue.

TABLE C-5
SUMMARY OF STYRENE OXIDE CARCINOGENICITY STUDIES WITH RATS

Strain	Route	No/ group	Exposure level	Schedule	Duration	Tumor site/type	Comments	Reference
Sprague-Dawley	Gavage	40 ♂ 40 ♀	50, 250 mg/kg/d	4-5d/wk	52 weeks. 104 weeks observation	dose-related increase in fore-stomach tumors (papillomas and squamous cell carcinomas)	non-neoplas-tic fore-stomach lesions in treated animals	Maltoni *et al*, 1979
Sprague-Dawley	Gavage	40 ♂ 40 ♀	50, 250 mg/kg/d	4-5d/wk	52 weeks. lifetime observation	dose-related increase in fore-stomach tumors (papillomas and squamous cell carcinomas)	non-neoplas-tic fore-stomach lesions in treated animals	Conti *et al*, 1988
BDIV	Gavage	42 ♂ 60 ♀	100-150 mg/kg/d	once per week	96 weeks. 24 weeks observation	forestomach papillomas and squamous cell carcinoma		Ponomarkov *et al*, 1984
Fischer 344	Gavage	52 ♂ 52 ♀	275, 500 mg/kg	3 per week	104 weeks. 3-4 weeks observation	forestomach papillomas and squamous cell carcinomas	non-neoplas-tic fore-stomach lesions in treated animals	Lijinsky, 1986

In a study by Ponomarkov and Tomatis (1984), styrene oxide was given in olive oil to pregnant BDIV rats at a dose of 200mg/kgbw on the 17th day of pregnancy, and the offspring were then given 96 weekly doses of 100-150mg/kgbw. A statistically significant increase in forestomach tumours, together with papillomas and other early changes indicative or chronic irritation (hyperkeratosis, hyperplasia, and dysplasia) was observed in the styrene-

TABLE C-6
Summary of Styrene Oxide Carcinogenicity Studies with Mice

Strain	Route	No/ group	Exposure level	Schedule	Duration	Tumor site/type	Comments	Reference
C3H	Dermal	30-40	5, 10%	3 per week	lifetime	none		Weil *et al*, 1963
Swiis-Millerton	Dermal	30 ♂	10%	3 per week	lifetime	none		Van Duuren *et al*, 1963
B6C3F1	Gavage	52 ♂ 52 ♀	375, 750 mg/kg	3 per week	104 weeks. 3-4 weeks observation	forestomach papillomas and squamous cell carcinomas in treated animals. Increased liver neoplasms in low dose males	non-neoplas-tic fore-stomach lesions in treated animals	Lijinsky, 1986

oxide treated animals. There were no other statistically significant differences in the incidence of other tumour types.

Lijinsky (1986) reported a study in which styrene oxide was given to rats and mice in corn oil by gavage. Male and female Fischer 344 rats were given 275 or 550mg/kgbw, and B6C3F1 mice were given 375 or 750mg/kgbw styrene oxide, 3 times per week, for up to 104 weeks. Consistent with the results of previous gavage studies, a high incidence of squamous cell carcinomas, papillomas and other non-neoplastic lesions indicative of irritation were found in the forestomach of styrene oxide-treated animals. There was no indication of a dose-related increase in tumours in any other organ or tissue in either male or female rats or mice.

In contrast to the results obtained in gavage studies, long-term dermal studies in which styrene oxide was applied three times per week to the skin as a 5% or 10% solution in either acetone or benzene showed no indication of an oncogenic response in either C3H or Swiss-Mullerton mice (Van Duuren, 1963; Weil, 1963).

To summarise, long-term oral studies have consistently shown a high incidence of forestomach neoplasias in rodents given high doses of styrene oxide by gavage. The response in the rodent forestomach has been associated with intense chronic irritation at the site of application, and there has been no indication of systemic oncogenic effects related to treatment with styrene oxide. The absence of an oncogenic response at the site of skin application in long-term dermal studies indicates that styrene oxide is not a potent carcinogen when given at non-irritatant dose levels, and furthermore suggests that chronic irritation may be an important factor in the development of forestomach tumours in the oral

studies. This suggestion is further substantiated by the low degree of forestomach DNA-binding in recent studies by Cantoreggi and Lutz (1992), who concluded that the styrene oxide-induced forestomach tumours were therefore probably the result of strong tumour promotion by regenerative hyperplasia.

In general, the relevance of rodent forestomach tumours for human risk estimations has been quite controversial (Webster and Kroes, 1988; Frederick and Chang-Mateu, 1990). Squamous cell carcinomas of the forestomach have been observed in many long-term oral or gavage studies with rodents. The risk estimations are complicated by the absence of a homologue for the forestomach in man, and it is important to understand the mechanism of action in estimating human risks. Discrimination between a mutagenic event and cell proliferation as primary mechanisms of action may be important to low dose risk assessments, since a non-linear dose-response curve (if not a threshold) is likely to exist where irritation, toxicity and regenerative hyperplasia are primary effects.

The development of forestomach tumours in styrene oxide gavage studies is of even greater uncertainty when making risk estimations for man in which styrene oxide occurs only at low levels as a transient, intermediate metabolite of styrene in which case there is little possibility of direct exposure of the stomach epithelium to styrene oxide.

APPENDIX D. MUTAGENICITY

D.1. MUTAGENICITY STUDIES

The potential mutagenicity of styrene and its intermediate metabolite, styrene-7,8-oxide (Styrene Oxide), has been the subject of numerous studies in a variety of assay systems (both prokaryotic and eukaryotic), as reviewed recently by Preston (1990a). There is an extensive literature on the mutagenicity testing of styrene and styrene oxide in the Ames bacterial mutagenicity assay (Milvy and Garro, 1976; Vainio *et al*, 1976; de Meester *et al*, 1977a,b, 1981; Watabe *et al*, 1978a, 1978b, 1982; Busk, 1979; El-Tantawy and Hammock, 1980; Yoshikawa *et al*, 1980; and Dunkel *et al*, 1985). The study by Dunkel *et al* (1985) was particularly significant since styrene was studied in 5 strains of Salmonella (TA98, TA100, TA1535; TA1537, and TA1538) at four different laboratories using six different exogenous metabolic activation systems derived from three species. The dose range for styrene was 0.3 to 333.3µg per plate. No mutagenic responses were obtained at any dose level for any of the *Salmonella* tester strains, with or without metabolic activation. These results are particularly important because of the fact that four independent laboratories reached identical conclusions using the same test protocols.

Styrene oxide is positive in the Ames test both in the presence or in the absence of metabolic activation, and its effectiveness seems to be enhanced by inhibitors of detoxification mechanisms. The (R) enantiomer of styrene oxide is apparently more mutagenic in the Ames test than the (S) enantiomer, with the racemic mixture intermediate between the two (Pagano *et al*, 1982). The various styrene oxide metabolites produced via epoxide hydratase (e.g., styrene glycol, mandelic acid, benzoic acid, phenylglyoxylic acid and hippuric acid), as well as thioether conjugates resulting from reaction of styrene oxide with glutathione have been shown to be non- mutagenic in the Ames bacterial assay (McCann and Ames, 1975; Milvey and Garro, 1976; King *et al*, 1979; Pagano *et al*, 1982)

The mutagenic potential of styrene has also been tested in several eukaryotic assay systems. Loprieno *et al* (1976) evaluated the mutagenic potential of styrene in yeast *in vitro* and in a host-mediated assay. With concentrations up to 100mmol, styrene produced no increases in point mutations or gene conversions *in vitro*. In the host-mediated assay in which Swiss albino mice were given a single i.p. dose of 1,000mg/kgbw styrene, gene conversion was increased at all sampling points, but there was no increase in point mutations. Styrene oxide produced dose-related increases in both point mutations and gene conversions over a concentration range of 0-20mmol. It is important to note that gene conversion has not been a consistent indicator of mutagenicity, since positive responses

have been obtained with a variety of chemicals when other assays have been negative. A positive response was reported with Drosophila using sex-linked recessive lethals as the end point (Donner *et al*, 1979), but the study was small in size and the results are therefore non-conclusive. Another Drosophila study by Sorsa *et al* (1978) showed no significant increase in sex-linked recessive lethals with either styrene or styrene oxide, but the methods and data were insufficiently reported to allow definitive conclusions.

Loprieno *et al* (1978) tested styrene and styrene oxide for the induction of forward mutations at the HGPRT locus in Chinese hamster V79 cells. Styrene was non-mutagenic at concentrations of 8.5, 17 or 51mmol (S10 metabolic activation system included), while styrene oxide was mutagenic at concentrations of 4.2, 8.5 and 17mmol in the absence of S10. Bonatti *et al* (1978) similarly reported that styrene oxide was mutagenic in Chinese hamster ovary cells at the HGPRT locus in studies which showed that the time of expression is important for maximizing the response. The mutagenic potential of styrene and styrene oxide in V79 Chinese hamster cells has also been evaluated using the isolated-perfused rat liver as a metabolizing system (Beije and Jenssen, 1982). The authors concluded that styrene was either very weakly mutagenic or non-mutagenic, while styrene oxide was non-mutagenic (styrene oxide levels were very low in the liver perfusate).

Overall, Preston (1990a) has concluded that styrene is not mutagenic in *in vitro* assays without metabolic activation. In the presence of metabolic activation, styrene is either weakly mutagenic or non-mutagenic. Styrene oxide is mutagenic in both prokaryotic and eukaryotic systems in the presence or absence of metabolic activation. It is difficult to predict *in vivo* effects from *in vitro* assays which utilize exogenous metabolic activation systems, and the *in vivo* significance of styrene oxide as a mutagenic intermediate metabolite of styrene remains unclear.

D.2. CYTOGENETIC STUDIES

A wide variety of assay systems have been utilized to determine the potential effects of styrene on chromosome number and structure, as recently reviewed by Preston (1990b). The endpoints that have been analyzed include chromosomal aberrations, sister chromatid exchanges, and aneuploidy. As noted by Preston (1990b), none of these assays can be used directly to estimate adverse health effects (somatic or genetic) of a particular agent, either because of the unsuitability of the cell type for direct extrapolation or because the end-point does not have known biological consequences. This is in agreement with the conclusions of the WHO-IPCS (1983) who were unable to assess the health significance of structural chromosomal aberrations in cells of individuals occupationally exposed to styrene.

Styrene appears to be either non-effective or marginally effective at inducing chromosome aberrations in Chinese hamster cells or human lymphocytes *in vitro*, while styrene oxide is considerably more effective than styrene at inducing chromosome aberrations in in vitro cell systems (Matsuoka *et al*, 1979; Turchi *et al*, 1981; Linnainmaa *et al*, 1978; Fabry *et al*, 1978; Norppa *et al*, 1981; Pohlova *et al*, 1985). Styrene and styrene oxide apparently can induce sister chromatid exchanges (SCE) in human lymphocytes *in vitro* (Norppa *et al*, 1980a, 1981, 1983; Norppa and Vainio, 1983; Pohlova *et al*, 1985). However, styrene did not cause an increase in SCE in an *in vitro* study with Chinese hamster cells, with or without metabolic activation, while styrene oxide was an effective inducer of SCE in this assay system (de Raat, 1978).

Various *in vivo* animal studies have also been conducted to determine if styrene has cytogenetic effects. Overall it appears that styrene does not induce chromosome aberrations in mouse, rat or Chinese hamster bone marrow cells, while styrene oxide does cause a low frequency of aberrations in mouse bone marrow cells, but not in rats or Chinese hamsters (Fabry *et al*, 1978; Loprieno *et al*, 1978; Meratoja *et al*, 1978a; Norppa *et al*, 1979; Norppa *et al*, 1980b; Sinha *et al*, 1983). Styrene has been reported to increase SCE in mouse bone marrow cells, alveolar macrophages, and regenerating liver cells (Conner *et al*, 1979; 1980a,b). However, another report indicated that styrene oxide did not induce SCE in Chinese hamster bone marrow cells (Norppa *et al*, 1979). According to Preston (1990b), the overall results of rodent *in vivo* cytogenetics studies are inconclusive, and further studies are needed.

In a recent study to evaluate the clastogenicity and SCE-inducing potential of styrene under carefully controlled conditions, Kligerman *et al* (1992) exposed B6C3F1 mice via inhalation to O, 125, 250, or 500ppm styrene vapours for 6 hours/day on 14 consecutive days. The animals in this study were exposed simultaneously with those of Morgan (1991). The two higher exposure concentrations present a severe challenge to B6C3F1 mice, as evidenced by substantial exposure-related mortalities. Peripheral blood, spleen and lungs were removed one day after the last exposure, and cells were cultured for the analysis of micronucleus induction, chromosome breakage, and SCE induction. Peripheral blood smears were also prepared in order to evaluate micronuclei in erythrocytes. No statistically significant exposure-related increases in the frequency of chromosomal aberrations were found in cultured splenocytes or lung cells, and there were likewise no significant increases in micronuclei splenocytes or erythrocytes in peripheral blood smears. However, a small concentration-related increase in SCE frequency was found in lymphocytes from spleen and peripheral blood as well as in cells from the lung. Based on results of these studies, the authors suggested that the reported increases in chromosome aberration frequencies in

peripheral blood lymphocytes of some groups of workers exposed occupationally to styrene may in fact be due to other factors in the workplace or environment. Moreover, the authors noted the fact that many epidemiological studies fail to show a significant increase in SCE and yet report increases in chromosome aberrations. Since SCE is generally considered to be a more sensitive indicator of S-dependent cytogenetic damage than chromosomal aberrations, the authors questioned whether the purported increases in chromosomal aberrations were due to styrene.

A number of human population monitoring studies have been reported, involving cytogenetics assessments in individuals occupationally exposed to styrene (Meretoja *et al*, 1977, 1978b; Fleig and Theiss, 1978; Hogstedt *et al*, 1979, 1983; Andersson *et al*, 1980; Theiss *et al*, 1980; Watanabe *et al*, 1981, 1983; Camurri *et al*, 1983; Dolmierski *et al*, 1983; Nordenson and Beckman, 1984; van Sittert and de Jong, 1985; Maki-Paakkanen *et al*, 1991; Norppa *et al*, 1991). Some of these studies report positive responses while others report negative responses. According to Preston (1990b), however, the published data fall short of allowing any risk estimation, and a variety of factors make these studies inclusive. Not only are the studies often fraught with technical problems (e.g. inadequate protocol, small sample size, only superficial control matching, and uncontrolled sources of variation), but also the exposure information on chemicals other than styrene is completely lacking. Therefore no firm conclusions can be drawn, and more definitively planned studies need to be conducted.

Sorsa *et al* (1991) recently published the results of a survey performed in 32 workshops in the reinforced plastics and composites industry. The mean 8-hour time-weighted average (TWA) styrene concentrations in the workshops were reported to be 43ppm (range 5-182ppm) among laminators, and 11ppm (range 1-133ppm) among other workers. Urinary mandelic acid plus phenylglyoxylic acid measurements showed mean values of 2.4mmol/l among laminators without respirators, and 1.3mmol/l for workers that used respirators. The workplace exposures were found to have no effects on cytogenetic parameters, chromosome aberrations, sister chromatid exchanges or micronuclei based on analyses of peripheral blood lymphocytes. Based on the results of this study, it seems clear that styrene has low potential to produce genotoxic effects in peripheral blood lymphocytes in workers exposed occupationally to styrene.

BIBLIOGRAPHY

Ames, B.N. and Gold, L.S (1990). Dietary carcinogens, environmental pollution and cancer: some misconceptions. Med. Oncol. and Tumor Pharm. 7; 1-17.

Andersson, H.C., Tranberg, E.A., Uggla, A.H. and Zetterberg, G. (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.

Andersen, M., and Ramsay, J. (1983). A physiologically-based description of the inhalation pharmacokinetics of styrene in rats and humans. In Developments in the Science and practice of Toxicology., (Hayes, A.W., Schnell, R.C., and Miya, T.S., Eds.), Elsevier. Amsterdam.

Andersen, M.E., Gargas, M.L. and Ramsey, J.C. (1984). Inhalation pharmacokinetics: evaluating systemic extraction, total *in vivo* metabolism, and the time course of enzyme induction for inhaled styrene in rats based on arterial blood: inhaled air concentration ratios. Toxicol. Appl. Pharmacol. 73: 176-187.

Bakke, O.M. and Scheline, R.R. (1970). Hydroxylation of aromatic hydrocarbons in the rat. Tox. Appl. Pharmacol. 16: 691-700.

Barale R. (1991). The genetic toxicology of styrene and styrene oxide : Mutation Res. 257, 107 - 126

Bardodej, Z. and Bardodejova, E. (1970). Biotransformation of ethyl benzene, styrene and alphamethylstyrene in man. Amer. Ind. Hyg. Assoc. J 31: 206-209.

Beije, B. and Jensson, 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. Interact. 39: 57-76.

Beliles, R.P. Butala, J.H., Stack, C.R. and Makris, S. (1985). Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fund App Tox 5:855-868.

Belvedere, G. and Tonsi, F. (1977). Kinetic behaviour of microsomal styrene monooxygenase and styrene expoxide hydratase in different animal species. Experientia 33(6): 708-709.

Berode, M., Boillat, M.A., Droz, P.O. and Guillemin, M., (1986). Effect of alcohol on the kinetics of styrene and its metabolites in volunteers and in workers. Appl. Ind. Hyg. 1: 25-28.

Bodell, W., Pongracz, K., Kaur, S., Burlingame, A.L., Lui, S.F. and Rappaport, S.M. (1990). Investigation of styrene oxide-DNA adducts and their detection in workers exposed to styrene. In mutation and the environment, Part C progress in clinical and biological research. Eds Mandelshorm, ML. and Albertini, R.I. Wiley-Liss Inc., 271-282.

Bonatti, S., A. Abbondandolo, G. Corti, R. Fiorio and A. Mazzaccaro (1978). The expression curve of mutants induced by styrene oxide at the HGPRT locus in V79 cells. Mutat. Res. 52: 295-300.

Bond, G., Bodner, K. and Cook, R. (1991). A critical review of eight studies involving nearly 50.000 workers. II. The epidemiology of styrene. The SIRC Review 2(1): 43-55.

Brenner, D.D., Jeffrey, A.M., Latriano, L., Wazneh, L., Warburton, D., Toor, M., Pero, R.W., Andrews, L.R., Walles, S. and Perera, F.P. (1991). Biomarkers in styrene exposed boatbuilders. Mutat. Res. 261, 225-236.

BUA (1990). Beratergremium fuer Umweltrelevante Altstoffe Report, No.48., "Styrene" Gesellschaft Deutscher Chemiker, VCH Weinheim.

Busk, L. (1979). Mutagenic effects of styrene and styrene oxide. Mutat. Res. 67: 201-208.

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.

Cantoreggi, S. and Lutz, W.K. (1992). Investigation of the covalent binding of styrene-7,8-oxide to DNA in rat and mouse. Carcinogenesis. 13(2): 193-197.

Cantoreggi, S. and Lutz, W.K. (in press). Covalent binding of styrene to DNA in rat and mouse. Carcinogenesis.

Cantoni, L., Salmona, M., Facchinetti, T., Pantarotto, C. and Belvedere, G., (1978). Hepatic and extrahepatic formation and hydration of styrene oxide *in vitro* in animals of different species and sex. Toxicol. Letters 2: 179-186.

Caperos, J.R., Humbert, B. and Droz, P.O. (1979). Exposition au styrene. Bilan de l'absorption, de l'excrétion et du métabolisme sur des sujets humains. Int. Arch. Occup. Environ. Health 42: 223-230.

Coggon, D., Osmond, C., Pannet, B., Simmonds, S., Winter, P.D. and Ackeson, D.E. (1987). Mortality of workers exposed to styrene in the manufacture of glass reinforced plastics. Scand. J. Work Environ. Health 13: 94-99.

Conner, M.K., Alarie, Y. and Dombroske, R.L. (1979). Sister chromatid exchange in regenerating liver and bone marrow cells of mice exposed to styrene. Toxicol Appl. Pharmacol. 50: 365-367.

Conner, M.K., Alarie, Y. and Dombroske, R.L. (1980a). Multiple tissue comparison of sister chromatid exchanges induced by inhaled styrene. In Genotoxic Effects of Airborne Agents (Tice, R.R., Costa, D.L., and Schaich, eds.) Plenum Press. N.Y. 433-441.

Conner, M.K., Alarie, Y. and Dombroske, R.L. (1980b). Sister chromatic exchange in murine alveolar macrophages, bone marrow, and regenerating liver cells induced by styrene inhalation. Toxicol. Appl. Pharmacol. 55: 37-42.

Conti, B., Maltoni, C., Perino, G. and Ciliberti, A. (1988). Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-Methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. Ann. N.Y. Acad. Sci. 534: 203-234.

Das, M., Dixit, R., Mushtaq, M., Srivastava, SP, and Seth, PK. (1981). Effect of styrene on hepatic mixed function oxidase, glutathione content and glutathione-S-transferase activity in rats. Drug. Chem. Toxicol. 4: 219-227.

De Meester, C., Poncelet, F., Roberfroid, M., Rondelet, J. and Mercier, M. (1977a). Mutagenic activity of styrene and styrene oxide. A preliminary study. Archives Internationales de Physiologie et de Biochimie 85: 398-399.

De Meester, C., Poncelet, F., Roberfroid, M., Rondelet J. and Mercier, M. (1977b). Mutagenicity of styrene and styrene oxide. Mutat. Res. 56: 147-152.

De Meester, C., Duverger-van Bogaert, M., Lamotte-Vandepare, M., Mercier, M. and Poncelet, F. (1981). Mutagenicity of styrene in the Salmonella typhimurium test system. Mut. Res. 90: 443-450.

Delbressine LPC, Van Bladeren, P.J., Smeets, F.L.M., and Seutter-Berlage, F. (1981). Stereoselective oxidation of styrene to styrene oxide in rats as measured by mercapturic acid excretion. Xenobiotica 11: 589-594.

de Raat, W.K. (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.

Dolmierski, R., Szczapani, M., Danielwicz-Garbalinski., G., Kunikowska, D., Mickiewicz, W., Chomizc, M. and Grosnicha, R. (1983). Mutagenic action of styrene and its metabolites. I. Chromosome aberration in persons exposed to the actions of styrene. Bull. Inst. Marit. Trop. Med. Gdynia 35: 89-93.

Donner, M., Sorsa, M. and Vainio, H. (1979). Recessive lethals induced by styrene and styrene oxide in Drosphila melanogastger. Mutat. Res. 67: 373-376.

Drummond, L., Caldwell, J. and Wilson, H.K. (1989). The metabolism of ethylbenzene and styrene to mandelic acid: stereochemical considerations. Xenobiotica 19: 199-207.

Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGreger, D. Mortelmans, K., Rosenkranz, H.S. and Simmon, V.F. (1985). Reproducibility of microbial mutagenicity assays. Testing of carcinogens and concarcinogens in Salmonella typhimurium and Escherichia coli. Environ. Mutagen. 7: (suppl. 5) 1-284.

ECETOC, (1989). European Chemical Industry Ecology and Toxicology Centre. DNA and Protein adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment. Monograph No. 13.

Ehrenberg, L. and Osterman-Golkar, S. (1980). Alkylation of Macromolecules for detecting mutagenic agents. Teratogen. Carcin. Mutat., 1, 105-127.

El-Tantawy, M.A. and Hammock, B.D., (1980). The effect of hepatic microsomal and cytosolic subcellular fractions on the mutagenic activity of epoxide-containing compounds in the salmonella assay. Mutat. Res. 79: 59-71.

Elovaara, E., Vainio, H., Pfaffli, P. and Collan, Y., (1979). Effects of intermittent styrene inhalation, ethalol intake and their combination on drug biotransformation in rat liver and kidneys. Med. Biol. 57:321.

Elovaara, E., Vainio, H. and Aitio, A. (1990). Pulmonary toxicity of inhaled styrene in acetone-phenobarbital- and 3-methylcholanthrene-treated rats. Arch. Toxicol. 64: 365-369.

Engstrom, J., Astrand, L. and Wigaeus, E. (1978a). Exposure to styrene in a polymerization plant: Uptake in the organism and concentration in subcutaneous adipose tissue. Scand. J. Work Environ. Health 4: 324-329.

Engstrom, J., Bjurstrom, R., Astrand, I. and Ovrum, P. (1978b). Uptake, distribution and elimination of styrene in man. Concentration in subcutaneous adipose tissue. Scand. J. Work Environ. Health 4: 315-323.

Engstrom, K. Harkonen, H., Killiokoski, P. and Rantane, J. (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.

Fabry, L., Leonard, A. and Robertfroid, M. (1978). Mutagenicity test with styrene oxide in mammals. Mutat. Res. 51: 377-381.

Farmer, P.B., Neumann, H.G. and Henschler, D. (1987). Estimation of Exposure to man to substances reacting covalently with macromolecules. Arch. toxicol., 60. 251-260.

Fielder, R.J. (1981). Toxicity Review 1; "Styrene" Health and Safety Executive U.K. (1981).

Filser, J.G., Kessler, W. and Schwegler, U. (1992). Study on the kinetics of styrene and styrene oxide in rats and mice. Draft report for ECETOC.

Filser, J.G. and Nolan, R. (in press). A physiological pharmacokinetic model for styrene and its metabolite styrene-7,8-oxide in mouse, rat, and man. Toxicol. Appl. Pharmacol.

Fleig, L., and Theiss, A.M. (1978). Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry. Scand. J. Work Environ. Health 4= 254-258.

Foureman, G.L., Harris, C., Guengerich, P. and Bend, J.R. (1989). Stereoselectivity of styrene oxidation in microsomes and in purified cytochrome P-450 enzymes from rat liver. J. Pharmacol. Exp. Ther. 248(2): 492-497.

Frederick, C.B. and Chang-Mateau, L.M. (1990). Contact site carcinogenicity Estimation of an upper limit for risk of dermal dosing site tumors based on oral dosing site carcinogenicity. In: Principles of Route-to-Route Extrapolation for Risk Assessment. Gerrity, T.R., and Henry, C.J., Eds. Elsevier Science Publishing Co., Inc.

Guillemin, M.P. 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: 873-879.

Guillemin, M.P. and Bauer, D. (1979). Human exposure to styrene. III. Elimination kinetics of urinary mandelic and phenylglyoxylic acids after single experimental exposure. Int. Arch. Occup. Environ. Health 44: 249-263.

Guillemin, M.P. and Berode M. (1988). Biological monitoring of styrene. A review. Am. Ind. Hyg. Assoc. J. 49: 497-505.

Hemminiki, K. (1983). Reaction of methylnitrosourea, epichlorohydrin, styrene oxide and acetoxyacetylaminofluorene with polyamino acids. Carcinogenesis, 4, 1-3.

Hemminki, K. (1986a). Binding of styrene oxide to amino acids, human serum proteins and haemoglobin. Arch. Toxicol Suppl. 9. 286-290.

Hemminki, K. (1986b). Binding of styrene oxide to amino acids, human serum proteins and haemoglobin. In monitoring of occupational genotoxicants, Alan R. Liss, Inc. 159-168.

Hemminki, K., Forsti, A. and Savela, K. (1990). Synthesis and stability of 2-deoxyguanosine-3'-mono-phosphate adducts of dimethylsulfate, ethylene oxide and styrene oxide. Chem. Biol. Interact. 75, 281-282.

Hiratsuka, A., Aizawa, T., Ozawa, N., Isobe, M., Watabe, T. and Takabatake, E. (1982). The role of epoxides in the metabolic activation of styrene to mutagens. In Proceedings of the 8th Symposium on Environmental Pollutants and Toxicology, October 8-9, 1981, Sendai, Japan. Eisei Kagaku 28: P.34.

Hodgson, J.T. and Jones, R.D. (1985). Mortality of styrene production, polymerization and processing workers at a site in Northwest England. Scand. J. Work Environ. Health 11: 347-352.

Hogstedt, B., Hedner, K., Mark-Vendel, E., Mitelman, F., Schutz, A. and Skerfving, S. (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., Gullberg, B., Mitelman, F., Pero, R.W., Skerfving, S. and Welinder, H. (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.

HSE (1991). Health and Safety Exective. Committee on carcinogenicity. Qualitative risk estimation. HMSO London.

Ikeda, M., Ohtsuji, H. and Imamura, T. (1972). *In vivo* suppression of benzene and styrene oxidation by co-administered tolune in rats and effects of phenobarbital. Xenobiotica 2: 101-106.

Ikeda, M. and Hirayama, T. (1978). Possible metabolic interaction of styrene with organic solvents. Scand J Work Environ Health 4: 41-46.

Imbriani, M., Ghittori, S., Pezzagno, G. and Capodaglio. (1985). Toluene and styrene in urine as biological exposure indices. Ann. Conf. Ind. Hyg., 12, 351-355.

Jahnke, G.D., Thompson, C.L., Walker, M.P., Gallagher, J.E., Lucier, G.W. and DiAugustine, R.P. (1990). Multiple DNA adducts in lymphocytes of smokers and nonsmokers determined by ^{32}P-postlabelling analysis. Carcinogenesis, 11, 205-211.

James, S.P., and White, D.A. (1967). The metabolism of phenethylbromide and styrene and styrene oxide in the rabbit and rat. Biochem. J. 104: 914-921.

Jersey G.C., Balmer, M.F., Quast, J.F., Park, C.N., Schuetz, D.J., Beyer, J.E., McCollister, S. and Rampy, L. (1978). Two year chronic inhalation toxicity and carcinogenicity study of monomeric styrene in rats - Final report CMA No. : Sty 1.1-Tox.-Inh. Chemical manufacturer Association.

Kaur, S., Hollander, D., Haas, R. and Burlinghame, A.L. (1989). Characterisation of structural xenobiotic modification in protein by high sensitivity tandem mass spectrometry. Human haemoglobin treated in vitro with styrene-7,8-oxide. J. Biol. Chem., 264, 16981-16984.

Kessler, W., Jiang, X. and Filser, J.G. (1990). Direct determination of styrene-7.8-oxide in blood by gas chromatography with flame ionization detection. J. of Chromatography 534: 67-75.

King, M.T., Beikirch, K., Eckhardt, K., Gocke, E. and Wild, D. (1979). Mutagenicity studies with X-ray contrast media, analgesics, antipyretics, antirheumatics and some other pharmaceutical drugs in bacterial Drosphila and mammalian test systems. Mutat. Res. 66: 33-43.

Kligerman, A.D., Allen, J.W., Bryant, N.F., Campbell, J.A., Collins, B.W., Doerr, C.W., Erexson, G.L., Kwanyuen, P. and Morgan, D.L. (1992). Cytogenetic studies of mice exposed to styrene by inhalation. Mutat. Res., 280, 35-43.

Korn, M., Wodarz, R., Drysch, K. and Schmahl, F.W. (1987). Stereometabolism and styrene in man: Urinary excretion of chiral styrene metabolites. Arch. Toxicol. 60: 86-88.

Lambotte - Vandepaer, M., Duverger-van Bogaert M., De Meester, C., Noel, G., Poncelet, F., Roberfroid, M. and Mercier M. (1979). Styrene induced modifications of some rat liver enzymes involved in the activation and inactivation of xinobiotics. Biochem. Pharmacol. 28: 1653-1659.

Langvardt, P.W. and Nolan, R.J. (1991). Determination of intact styrene-7,8-oxide using automated gas chromatography - mass spectrometry. J. Chromatography 567: 93-103.

Lijinski, W. (1986). Rat and mause forestomach tumors induced by chronic oral administration of styrene oxide. J. Natl. Cancer Inst. 77: 471-476.

Linnainmaa, K., Meretoja, T., Sorsa, M. and Vainio, H. (1978). Cytogenetic effects of styrene and styrene oxide. Mutat. Res. 58: 277-286.

Liu, S.F., Rappaport, S.M., Pongracz, K. and Bodell, W.J. (1988). Detection of styrene oxide-DNA adducts in lymphocytes of a worker exposed to styrene. In: methods for detecting DNA damaging agents in humans. Applications in cancer epidemiology and prevention. IARC Scientific Publication No 89. IARC Lyon, 217-222.

Lof, A., Gullstrand, E. and Nordqvist, B. (1983). Tissue distribution of styrene, styrene glycol and more polar styrene metabolites in the mouse. Scan. J. Work Environ. Health 9: 419-430.

Lof, A., Gullstrand, E., Lundgren, E. and Nordqvist, M.B. (1984). Occurence of styrene-7,8-oxide and styrene glycol in mouse after the administration of styrene. Scan. J. Work Environ. Health 10: 179-187.

Lof, A., Lundgren, E. and Byfalt Nordzvist, M. (1986a). Kinetics of styrene in workers from a plastics industry after controlled exposure: A comparison with subjects not previously exposed. Brit. J. Indust. Med. 43: 537-543.

Lof, A., Lundgren, E., Nydahl, E.-M. and Byfalt Nordzvist, M. (1986b). Biological monitoring of styrene metabolites in the blood. Scand. J. Work Environ. Health 12: 70-74.

Loprieno, N., Abbondolo, A., Barale, R., Barocelli, S., Bonatti, S., Bronzetti, G., Cammellini, A., Corsi, C., Corti, G., Fezza, D., Leporini, C., Mazzaccaro, A., Nieri, R., Rosellini, D. and Rossi, A.M. (1976). Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide. Mutat. Res. 40: 317-324.

Loprieno, N., Presciuttini, S., Sbrana, L., Stretti, G., Zaccaro, L., Abbondandolo, A., Bonatti, S., Fiorio, R. and Mazzaccaro, A. (1978). Mutagenicity of industrial compounds VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyse. Scand. J. Work Environ. Health 4(2): 169-178.

Lutz, W.K. (1986). Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens. J. Cancer Res. Clin. Oncol. 112 85-91

Lutz, W.K. (1992). Investigation of the adduct formation between styrene or styrene-7,8-oxide and deoxyribonucleic acid (DNA) in rats, mice and in vitro. Study performed for ECETOC.

Lutz, W.K. and Contoreggi, S. (in press). An improved ^{32P}-Postlabelling assay for detection and quantitation of styrene-7,8-oxide-DNA adducts.

Maki-Paakkanen, J., Walles, S., Osterman-Golkar, S. and Norppa, H. (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. Environ. Mutagen. 17: 27-31.

Malonova, H. and Bardodej, Z. (1983). Urinary excretion of mercaptures or a biological indicator of exposure to electrophatic agents. J. Hyg. Epid. Microbiol. Immunol. 27: 319-328.

Maltoni, C., Failla, G., and Kassapidis, S. (1979). First experimental demonstration of the carcinogenic effects of styrene oxide. Med. Lavoro. 5: 358-362.

Maltoni, C., Ciberti, A. and Carrietti, D. (1982). Experimental contributions in identifying brain potential carcinogens in the petrochemical industry. Ann. N.Y. Acad. Sci. 381: 216-249. Cited In: NCI 1985.

Marniemi, J. and Parkki, M.G. (1975). Radiochemical assay of glutathione S-expoxide transferase and its enhancement by phenobarbital in rat liver *in vivo*. Biochem. Pharmacol 24: 1569-1572.

Matanoski, G.M. and Schwarts, L. (1987). Mortality of workers in styrene-butadiene polymer production. J. Occup. Med. 29: 675-680.

Matsuoka, A., Hayashi, M. and Ishidate, M., Jr. (1979). Chromosome aberration tests on 29 chemicals combined with S9 mix *in vitro*. Mutat. Res. 66: 277-290.

McCann, J. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72: 5135-5139.

Meinhardt, J.J., Lemen, R.A., Crandall, M.S. and Young, R.J. (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Scand. J. Work Environ. Health. 8, 250-259.

Mendrala, A.L., Langvardt, P.W., Nitschke, K.D., Quast, J.F. and Nolan, R.J. (1992). *In vitro* kinetics of styrene and styrene oxide metabolism in rat, mouse and human. Arch. Toxicol. (in press).

Meretoja, T., Vainio, H., Sorsa, M. and Harkonen H. (1977). Occupational styrene exposure and chromosomal aberrations. Mutat. Res. 56: 193-197.

Meretoja, T., Vainio, H. and Jarventaus, H. (1978a). Clastogenic effect of styrene exposure on bone marrow cells of rat. Toxicol. Letters 1: 315-318.

Meretoja, T., Jarventaus, H., Sorsa, M. and Vainio, H. (1978b). Chromosome aberrations in lymphocytes of workers exposed to styrene. Scand. J. Work Environ. Health 4(Suppl.2): 259-264.

Milvey, P. and Garro, A.J. (1976). Mutagenic activity of styrene oxide (1,2-epoxyethyl-benzene), a presumed styrene metabolite. Mutat. Res. 40: 15-18.

Morgan, D. (1991). National Institute of Environmental Health Sciences. Personal Communication.

Nicholson, W.J. Selikoff, N. and Serdman, H. (1978). Mortality experience of styrene-polystyrene polymerization workers. Scand. J. Work Environ. Health. Suppl. 2: 247-252.

NCI. (1978). National Cancer Institute Bioassay of a solution of B-nitrostyrene and styrene for possible carcinogenicity. CAS No. 102-965. CAS No. 100-42-5. NCI-CG-TR-170. Bethesda, MD: US Dept of Health, Education and Welfare, National Institute of Health Report No. 79-1726.

NCI. (1979). National Cancer Institute Bioassay of styrene for possible carcinogenicity. CAS No. 100-42-5. NCI-CG-TR-185. Bethesda, MD: US Dept. of Health, Education and Welfare, National Institutes of Health Report No. 79-1741.

Nolan, R.J., Langvardt, P.W., Markham, D.A., and Smith, F.A. (1991). Time-Course of Styrene Oxide in Whole Blood From Rats Given a Single Oral Dose of Styrene Oxide. The Dow Chemical Company, Final Report for SERC.

Nordenson, I., and Beckman, L. (1984). Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene. Human Heredity 34: 178-182.

Nordqvist, M., Lof, A., Osterman-Golkar, S. and Walles, S.A.S. (1985). Covalent binding of styrene and styrene-7,8-oxide to plasma proteins, haemoglobin and DNA in the mouse. Chem. Biol. Interact. 55, 63-73.

Norppa, H., Elovaara, E., Husgafvel-Pursiainen, K., Sorsa M. and Vainio H. (1979). Effects of styrene oxide on chromosome aberrations, sister chromatic exchange and hepatic drug biotransformation in Chinese Hamsters *in vivo*. Chem. Biol. Interact. 26: 305-315.

Norppa, H., Sorsa, M., Pfaffli, P. and Vainio H. (1980a). Styrene and styrene oxide induce SCEs and metabolized in human lymphocyte cultures. Carcinogenesis 1: 357-361.

Norppa, H., Sorsa, M., and Vainio, H. (1980b). Chromosomal aberrations in bone marrow of Chinese Hamsters exposed to styrene and ethanol. Toxicol. Letters 5: 241-244.

Norppa, H., Hemminki, K., Sorsa, M. and Vainio H. (1981). Effect of monosubstituted epoxides on chromosome aberrations and SCE in cultured human lymphocytes. Mutat. Res. 91: 243-250.

Norppa, H., and Vainio, H. (1983). Genetic toxicology of styrene and some of its derivatives. Scand. J. Work Environ. Health 9: 108-114.

Norppa, H., Jarventaus, H., Kubiak, R., Maki-Paakansen, L., Nylander, P., Pfaffli, K., Pekari, A. and Sorsa, M. (1991). Chromosome aberrations, sister chromatid exchanges, and micronuclei in blood lymphocytes of Finnish reinforced plastics workers exposed to styrene. Environ. Molec. Mutagen. 17 (Suppl. 19): 56 (Abstract).

Oesch, R. (1972). Mammalian expoxide hydrases: inducible enzymes catalysing the inactivation of carcinogenic and cytoxic metabolites derived from aromatica and olefinic compounds. Enobiotica 3(5): 305-340.

Ohtsuji, H. and Ikeda, M. (1971). The metabolism of styrene in the rat and the stimulatory effect of phenobarbital. Toxicol. Appl. Pharmacol. 18: 321-328.

Okun, A.H., Beaumont, J.J., Meinhardt, T.J. and Crandall, M.S. (1985). Mortality patterns among styrene-exposed boatbuilders. Am. J. Indust. Med. 8: 193-205.

Osterman-Golkar, S. (1991). Blood protein adducts as markers of exposure to toxic chemicals (abstract). Soc. of Toxicol. Meeting; Dallas 1991.

Osterman-Golkar, S. (1992). Investigation of the adduct formation between styrene or styrene metabolites and haemoglobin or blood proteins in rats and mice (In vitro and In vivo). Study performed for ECETOC.

Ott, M.G., Kolesar., R.C., Scharnweber, H.C., Schneider, E.J. and Venable, J.R. (1980). A mortality survey of employees engaged in the development or manufacture of styrene-based products. J. Occup. Med. 22: 445-460.

Pacifici, G.M., Boobis, A.R., Brodie, M.J., McManus, M.E. and Davis, D.S. (1981). Tissue and species differences in enzymes of epoxide metabolism. Xenobiotica 11(2): 73-79.

Pacifici, G.M., Warholm, M., Guthenberg, C., Mannervik, B. and Rane, A. (1987). Detoxification of styrene oxide by human liver glutathione transferase. Human Toxicol. 6:483-489.

Pagano, D.A., Yagen, B., Hernandez, O., Bend, J.R. and Zeiger, E. (1982). Mutagenicity of (R) and (S) styrene-7,8-oxide and the intermediary mercapturic acid metabolites formed from styrene-7,8-oxide. Environ. Mutagen. 4: 575-584.

Pantarotto, C., Fanelli, R., Bidoli, F., Morazzoni, P., Salmona M. and Szczawiska K. (1978). Arene oxides in styrene metabolism, a new perspective in styrene toxicity. Scand. J. Work Environ. Health 4(Suppl.) 2): 67-77.

Pantarotto, C., Fanelli, R., Belletti, I. and Bidoli F. (1980). Determination of styrene in biological specimens by gas chromatography-selected ion monitoring: Distribution in mice. Anal. Biochem. 105: 340-347.

Pantarotto, C., and Blonda, C. (1984). Covalent binding to proteins as a mechanism of chemical toxicity. Arch. Toxicol. Suppl. 7: 208-218.

Parkki, M.G., Marniemi, J. and Vainio, H. (1976). Action of styrene and its metabites styrene oxide and styrene glycol on activities of xenbiotic biotransformation enzymes in rat liver *in vivo*. Toxicol. Appl. Pharmacol. 38: 59-70.

Pfaffli, P., Vainio, H. and Hesso, A. (1979). Styrene and styrene oxide concentrations in the air during the lamination process in the reinforced plastics industry. Scand. J. Work Environ. Health 5: 158-161.

Pfaffli, P., Hesso, A., Vainio, H. and Hyvonen, M. (1981). 4-Vinylphenol excretion suggestive of arene oxide formation in workers occupationally exposed to styrene. Toxicol. Appl. Pharmacol. 60: 85-90.

Philips, D.H., Hewer, P.C. and Grover, P.L. (1986). Aromatic DNA adducts in human bone marrow and peripheral blood leukocytes. Carcinogenesis, 7, 1881-1887.

Plotnick, H.B. and Weigel, W.W. (1979). Tissue distribution and excretion of 14C-styrene in male and female rats Res. Commun. Chem. Pathol. Pharmocol. 24: 515-524.

Pohlova, H., Rossner, P. and Stram, R.J. (1985). Cytogenetic analysis of human peripheral blood lymphocytes in culture exposed *in vitro* to styrene and styrene oxide. J. Hyg. Epidemiol. Microbiol. and Immunol. 29: 269-274.

Pongracz, K., Kaur, S., Burlingame, A.L. and Bodell, W.J. (1992). Identification of N^2-substituted 2'-deoxyguanosine-3'-phosphate adducts detected by ^{32P}-postlabelling of styrene-oxide-treated DNA. Carcinogenesis. 13, 315-319.

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.

Ponomarkov, V. and Tomatis, L. (1984). A carcinogenicity study of styrene-7,8 oxide in rats. Cancer lett. 24: 95-101.

Preston, R.J. (1990a). The potential mutagenicity of styrene and its metabolites; The SIRC Review. 1(1): 25-31.

Preston, R.J. (1990b). Styrene and its metabolites: A discussion of results from cytogenetic assays. The SIRC Review. 1(2): 23-37.

Quast, J.C., Humiston, C.G. and Kalvins, R.V. (1979). Results of a toxicity study of monomeric styrene administered to beagle dogs by oral intubation for 19 months. Final Report. Toxicology Research Laboratory, Health and Environmental Sciences. The Dow Chemical Company, Midland, MI.

Ramsey, J.C. and Young J.D. (1978). Pharmacokinetics of inhaled styrene in rats and humans. Scand. J. Work Environ. Health 4(Supp.2): 84-91.

Ramsey, J.C. and Andersen, M.E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol. Appl. Pharmacol., 73: 159-175.

Reddy, M.V., Kenny, P.C. and Randerath, K. (1990). ^{32}P-assay of DNA adducts in white blood cells and placenta of pregnant women: Lack of residential wood combustion-related adducts but presence of tissue specific endogenous adducts. Teratogenesis carcinogenesis mutagenesis. 10, 373-387.

Romano, M., Garattini, E. and Salmona, M. (1983). Perinatal development of styrene monooxygenase and epoxide hydrolase in rat liver microsomes and nuclei. Chem. Biol. Interactions 47: 213-222.

Romano, M., Garattini, E., and Salmona, M. (1985). Perinatal development of cytochrome P-450, cytochrome C reductase, aryl hydrocarbon hydroxylase, styrene monooxygenase, and styrene epoxide hydrolase in rabbit liver microsomes and nuclei. Dev. Pharmacol. Ther. 8: 232-242.

Rothman, N., Poirier, M.C., Baser, M.E., Hansen, J.A., Gentile, C., Bowman, E.D. and Strickland, P.T. (1990). Formation of Polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells during consumption of charcoal-broiled beef. Carcinogenesis, 11, 1241-1243.

Ryan, A.J., James, M.O., Ben-Zvi, Z. Law, F.C.P. and Bend, J.R. (1976). Hepatic and extrahepatic metabolism of 14C-Styrene oxide. Environ. Health Perspect. 17: 135-144.

Sandell, J., Parkki, J., Aitio, A. (1978). Effects of inhalaltion and cutaneous exposure to styrene on drug metabolizing enzymes in the rat. Int. Congr. Ser. 440: 177.

Savela, K., Hesso, A. and Hemminki, K. (1986). Characterization of reaction products between styrene oxide and deoxynucleotides and DNA. Chem. Biol. Interact., 60, 235-246.

Savela, K. and Hemminki, K. (1991). DNA adducts in lymphocytes and granulocytes of smokers and nonsmokers detected by ^{32}P postlabelling assay. Carcinogenesis, 12, 503-508.

Sepai, O., Anderson, D., Street, B., Bird, I., Farmer, P.B. and Bailey, E. (1992). The monitoring of exposure to styrene oxide by GC-MS analysis of phenylhydroxylethl esters in haemoglobin. Submitted for publication.

Seutter-Berlage, F., Delbressine, L.P.C., Smeets, F.L.N. and Ketelaars, H.C.J. (1978). Identification of three sulphur-containing urinary metabolites of styrene in the rat. Xenobiotica, 8: 413-418.

Sinha, A.K., Jersey, G.C., Linscombe, V.A., Adams, R.L., Mueller, A.M. and McClintock, M.L. (1983). Cytogenetic evaluation of bone marrow cells from rats exposed to styrene vapor for one year. Fundam. Appl. Toxicol. 3: 95-98.

SIRC, Styrene Information and Research Center (1991). Classifying the carcinogenicity issue. The Styrene Research Programme. SIRC Review, July 1991.

Sorsa, M., Laarnanen, I. and Vainio, H. (1978). Mutagenicity of styrene nad styrene oxide in Drosophila tests. Mutat. Res. 52: 266-267.

Sorsa, M., Anttila, A., Jarventaus, H., Kubiak, R., Norppa, H., Nylander, L., Pekari, K., Pfaffli, P. and Vainio, H. (1991). Styrene revisited - Exposure assessment and risk estimation in reinforced plastics industry. New Horizons in Biological Dosimetry. Prog. Clin. Biol. Res. 37: 187-195.

STEP (1988). EEC Scientific and Technology for Environmental Protection. Biomonitoring of human populations exposed to genotoxic environmental chemicals. Commission of the European Communities, Directorate-General for Science, Research and Development.

Stewart, R.D., Dodd, H.C., Baretta, E.D. and Schaffer, A.W. (1968). Human exposure to styrene vapor. Arch. Environ. Health 16: 656-662.

Teramoto, K. and Horiguchi, S. (1979). Absorption, distribution and elimination of styrene in man and experimental animals. Arh. Hig. Rada. Toksikol. 30: 431-439.

Teramoto, K. and Horiguchi, S. (1981). Distribution, elimination and retention of styrene in rats. J. Toxicol. Sci. 6: 13-18.

Theiss, A.M., Schwegler, H. and Fleig, I. (1980). Chromosome investigations in lymphocytes of workers employed in areas in which styrene-containing unsaturated polyester resins are manufactured. Am. J. Industrial Med. 1: 205-210.

Turchi, G. Bonatti, S., Citti, L., Gervasi, P.G. and Abbondandolo, A. (1981). Alkylating properties and genetic activity of 4-vinylcyclohexene metabolites and structurally related epoxides. Mutat. Res. 83: 419-430.

Vodicka, P. and Hemminki, K. (1988). Identification of alkylation products of styrene oxide in single and double stranded DNA. Carcinogenesis, 9, 1657-1660.

Vainio, H., Paakkonen, R, Ronnholm, K., Raunio, V. and Pelkonen, O. (1976). A study on the mutagenic activity of styrene and styrene oxide. Scand. J. Work Environ. Health 3: 147-151.

Vainio, H. and Zitting, A. (1978). Interaction of styrene and acetone with drug biotransformation enzymes in rat liver. Scand. J. Work Environ. Health 4(suppl. 2): 47-52.

Vainio, H., Jarvisalo, J. and Taskinen, E. (1979). Adaptive changed caused by intermittent styrene inhibition on xenobiotic biotransformation. Toxicol. Appl. Pharmacol. 49: 7-14.

Vainio H. (1991). "Nordic Criteria Documents from the Nordic Expert Group, 1990" Styrene p. 189.

Van Duuren, B.L. (1963). Carcinogenicity of epoxides, lactones and peroxy compounds. J. Natl. Cancer Inst. 31: 41-45.

van Sittert, N.J., and de Jong, G. (1985). Biomonitoring of exposures to potential mutagens and carcinogens in industrial populations. Food Chem.Toxicol. 23: 23-31.

Warholm, M., Guthenberg, C., Mannervik, B., Pacifici, G.M. and Rane, A. (1981). Glutathione S-transferase in human fetal liver. Act Chemical Scand. B35 : 225-227.

Watabe, T., Isobe, M., Sawahata, T., Yoshikawa, K., Yamada, S. and Takabatake, E. (1978a). Metabolism and muagenicity of styrene. Scan. J. Work Environ. and Health 4(2): 142-155.

Watabe, T., Ozawa, N. and Yashikawa, K. (1981). Stereochemistry in the oxidative metabolism of styrene by hepatic microsomes. Biochem. Pharmacol. 30: 1695-1698.

Watabe, T., Hiratsuka, A., Aizawa, T., Sawahata, T., Ozawa, N., Isobe, M. and Takabatake, E. (1982). Studies on metabolism and toxicity of styrene IV. 1-vinylbenzene 3;4-oxide, a potent mutagen formed as a possible intermediate in the metabolism *in vivo* to styrene to 4-vinylphenol. Mutat. Res. 93: 45-55.

Watanabe, T., Endo A., Sato, K., Ohtsuki, T., Kizuma, A. and Ikeda, M. (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. Environ. Mutagen. 5: 299-309.

Webster, P.W. and Kroes, R. (1988). Forestomach carcinogens: Pathology and relevance to man. Toxicologic. pathol. 16(2): 165-171.

Weil, C.S. (1963). Experimental carcinogenicity and acute toxicity of representative epoxides. Am. Ind. Hyg. Assoc. J. 24: 305-325.

WHO-IPCS (1983). Environmental Health Criteria Nr. 26, Styrene

Wigaeus, E., Lof, A., Bjurstrom, R. and Norqvist, M.B. (1983). Exposure to styrene. Uptake, distribution, metabolism, and elimination in man. Scand. J. Work Environ. Health 9: 479-488.

Wigaeus, E., Lof, A. and Nordqvist, M.B. (1984). Uptake, distribution, metabolism, and elimination of styrene in man. A comparison between single exposure and co-exposure with acetone. Brit. J. Indust. Med. 41: 539-546.

Wilson, H.K., Robertson, S.M. Waldron, H.A. and Gompertz, D. (1983). Effect of alcohol on the kinetics of mandelic acid excretion in volunteers exposed to styrene vapor. Br. J. Ind. Med. 40: 75-80.

Wolff M.S., Lorimer, W.V., Lilis, R. and Selikoff, I.J., (1978). Blood styrene and urinary metabolites in styrene polymerisation. Br. J. Ind. Med. 35: 318-329.

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. Brit. J. Ind. Med. 47:753-762.

Yoshikawa K., Isabe, M., Watabe, T. and Takabatake, E. (1980). Studies on metabolism and toxicity of styrene. III. The effect of metabolic inactivation by rat-liver S9 on the mutagenicity of Phenbyloxirane toward Salmonella typhimurium. Mutat. Res. 78: 219-226.

Young J.D., Ramsey, J.C., Blau, G.E., Karbowski, R.J., Nitschke, K.D., Slauter, R.W.A. and Braun, H.W. (1979). Pharmacokinetics of inhaled or intraperitoneally administered styrene in rats. In Dev. Toxicol. Environ. Sci. Toxicology and Occupational Medicine. Deichmann WB (Ed). Elsevier/North-Holland, New-York, Vol. 4 297-310.

MEMBERS OF THE TASK FORCE

HOFFMANN H. (Chairman)	BASF D - Ludwigshafen
MILLER R.	DOW CHEMICAL USA - Midland Michigan
MASON P.	BP CHEMICAL GB - London
WIEGAND H.	HUELS AG D - Marl
HUCKLE K.	SHELL NL - Den Haag
FEDTKE N.	HUELS D - Marl
LOMBARD A.	ATOCHEM F - Paris
POOLE A.	DOW EUROPE CH - Horgen
LODI S.	ENICHEM POLIMERI I - Milan
RAIMOND C.	LABOFINA B - Feluy
HAEBLER W.	ECETOC B - Brussels

MEMBERS OF THE ECETOC SCIENTIFIC COMMITTEE (Peer Review Committee)

W. F. TORDOIR (Chairman), Head of Occupational Health and Toxicology Division[a]	SHELL NL - Den Haag
H. VERSCHUUREN, (Vice-Chairman) Head of Toxicology Department[a]	DOW CHEMICAL CH - Horgen
O. C. BOECKMAN, Scientific Advisor	NORSK HYDRO N - Porsgrunn
N. G. CARMICHAEL, Toxicology Director Worldwide	RHONE-POULENC F - Lyon
H. DE HENAU, European Technical Centre Professional and Regulatory Services	PROCTER & GAMBLE B - Brussels
A. DE MORSIER, Head, Ecotoxicology	CIBA-GEIGY CH - Basel
P. A. GILBERT, Head, Environmental Relations	UNILEVER UK - Port Sunlight
I. J. GRAHAM-BRYCE, Head of Environmental Affairs	SHELL NL - Den Haag
B. HILDEBRAND, Director, Experimental Toxicology[a]	BASF AG D - Ludwigshafen
J. R. JACKSON, Director Medicine and Health Science	MONSANTO EUROPE B - Brussels
K. KUENSTLER, Head Biological Research	HENKEL D - Duesseldorf
H. LAGAST, Chief Medical Officer	SOLVAY B - Brussels
E. LOESER, Head of Institute of Industrial Toxicology	BAYER D - Wuppertal
R. MILLISCHER, Chief Toxicologist	ATOCHEM F - Paris
I. F. H PURCHASE, Director, Central Toxicology Laboratory	ICI UK - Macclesfield

a Stewards - responsible for primary peer review

LIST OF ECETOC PUBLICATIONS

MONOGRAPHS

No.	Title
No. 1	Good Laboratory Practice
No. 2	Contribution to Strategy for Identification and Control of Occupational Carcinogens
No. 2	Definition of a Mutagen, for 6th Amendment
No. 3	Risk Assessment of Occupational Chemical Carcinogens
No. 4	Hepatocarcinogenesis in Laboratory Rodents : Relevance for Man
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No. 6	Acute Toxicity Tests, LD_{50}(LC_{50}) Determinations and Alternatives
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No. 11	Eye Irritation Testing
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity)
No. 13	DNA and Protein Adducts: Evaluation of their Use in exposure Monitoring and Risk Assessment
No. 14	Skin Sensitisation Testing
No. 15	Skin Irritation
No. 16	Mutation Research, Special Issue: Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals

JACC REPORTS

No.	Title
No. 1	Joint Assessment of Commodity Chemicals, Melamine
No. 2	Joint Assessment of Commodity Chemicals, 1,4-Dioxane
No. 3	Joint Assessment of Commodity Chemicals, Methyl Ethyl Ketone
No. 4	Joint Assessment of Commodity Chemicals, Methylene Chloride
No. 5	Joint Assessment of Commodity Chemicals, Vinylidene Chloride
No. 6	Joint Assessment of Commodity Chemicals, Xylenes
No. 7	Joint Assessment of Commodity Chemicals, Ethylbenzene
No. 8	Joint Assessment of Commodity Chemicals, Methyl Isobutyl Ketone
No. 9	Joint Assessment of Commodity Chemicals, Chlorodifluoromethane
No. 10	Joint Assessment of Commodity Chemicals, Isophorone
No. 11	Joint Assessment of Commodity Chemicals, (HFA-132b) 1,2-Dichloro-1,1-Difluoroethane
No. 12	Joint Assessment of Commodity Chemicals, (HFA-124) 1-Chloro-1,2,2,2-Tetrafluoroethane
No. 13	Joint Assessment of Commodity Chemicals, (HFA-123) 1,1-Dichloro-2,2,2-Trifluoroethane
No. 14	Joint Assessment of Commodity Chemicals, (HFA-133a) 1-Chloro-2,2,2-Trifluoromethane
No. 15	Joint Assessment of Commodity Chemicals, (HFA-141B) 1-Fluoro 1,1-Dichloroethane
No. 16	Joint Assessment of Commodity Chemicals, (HCFC-21) Dichlorofluoromethane
No. 17	Joint Assessment of Commodity Chemicals, (HFA-142b) 1-Chloro-1,1,Difluoroethane
No. 18	Joint Assessment of Commodity Chemicals, Vinylacetate
No. 19	Joint Assessment of Commodity Chemicals, Dicyclopentadiene
No. 20	Joint Assessment of Commodity Chemicals, Tris-/Bis-/Mono-(2-ethylhexyl)phosphate
No. 21	Joint Assessment of Commodity Chemicals, Tris-(2-butoxyethyl)-phosphate
No. 22	Joint Assessment of Commodity Chemicals, Hydrogen Peroxide

TECHNICAL REPORTS

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man
No. 6	Formaldehyde Toxicology: an Up-Dating of the ECETOC Technical reports 1 and 2
No. 7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8	Biodegradation Testing: An Assessment of the Present Status
No. 9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11	Ethylene Oxide Toxicology and its Relevance to Man : An Up-Dating of ECETOC Technical Report n°5
No. 12	The Phototransformation of Chemicals in Water : Results of a Ring-Test
No. 13	The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on the Environment
No. 14	The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on Human Health
No. 15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16	A review of Recent Literature on the Toxicology of Benzene
No. 17	The Toxicology of Glycol Ethers and its Relevance to Man : An Up-Dating of ECETOC Technical Report n°4
No. 18	Harmonisation of Ready Biodegradability Tests
No. 19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20	Biodegradation Tests for Poorly-Soluble Compounds
No. 21	Guide to the Classification of Carcinogens, Mutagens and Teratogens Under the 6th Amendment
No. 22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24	The EEC 6th Amendment : Prolonged Fish Toxicity Tests
No. 25	Evaluation of Fish Tainting
No. 26	The Assessment of Carcinogenic Hazard for Human Beings Exposed to Methylene Chloride
No. 27	Nitrate and Drinking Water
No. 28	Evaluation of Anaerobic Biodegradation
No. 29	Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico- Chemical Properties, Tonnage and Use Pattern
No. 30(4)	Existing Chemicals : Literature Reviews and Evaluations
No. 31	The Mutagenicity and Carcinogenicity of Vinyl Chloride : A Historical Review and Assessment
No. 32	Methylene Chloride (Dichloromethane) : Human Risk Assessment Using Experimental Animal Data
No. 33	Nickel and Nickel Compounds : Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34	Methylene Chloride (Dichloromethane) : An Overview of Experimental Work Investigating Species, Differences in Carcinogenicity and their Relevance to Man
No. 35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36	Biomonitoring of Industrial Effluents
No. 37	Tetrachloroethylene : Assessment of Human Carcinogenic Hazard
No. 38	A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39	Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No. 40	Hazard Assessment of Chemical Contaminents in Soil
No. 41	Human Exposure to N-Nitrosmaines, Their Effects and a Risk Assessment for n-Nitrosodiethanolamine in Personal Care Products
No. 42	Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43	Emergency Exposure Indices for Industrial Chemicals
No. 44	Biodegradation Kinetics
No. 45	Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis
No. 46	EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47	EC 7th Amendment: 'Toxic to Reproduction' - Guidance on Classification
No. 48	Eye Irritation: Reference Chemicals Data Bank
No. 49	Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50	Estimating the Environmental Concentrations of Chemicals Using Fate and Exposure Models
No. 51	Environmental Hazard Assessment of Substances
No. 52	Styrene Toxicology Investigations on the Potential for Carcinogenicity