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Study on the Kinetics of Styrene and Styrene Oxide in Rats and Mice

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Report

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

Study performed for the
ECETOC Task Force Styrene

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

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* S = styrene; SO = styrene-7,8-oxide

1. SUMMARY

This report describes experiments on the kinetics of styrene (S*) and styrene-7,8-oxide (SO*) in Sprague-Dawley rats and B6C3F1 mice. The pharmacokinetic parameters for S and SO determined in this study permit quantitative description of the major pathways of S and SO in the organism of rat and mouse under various conditions of exposure.

For this purpose the two compounds were given at appropriate dose levels to the animals by inhalative, intravenous, intraperitoneal and oral route. In vivo data were generated using experimental approaches like closed chamber gas uptake studies, plasma level measurements, repeated pretreatment or inhibiton of metabolism prior to dosing. In vitro techniques were used to determine the partition coefficients for both compounds in systems comprising liquids/air; tissues/air and tissues/blood.

Results are used to calculate the kinetic parameters for both compounds. Summary tables of kinetic data for S and SO are presented in the report. The following conclusions are drawn:

A quantitative relation between exposure to S or to SO and occurrence of SO in the organism has been found.

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* In the whole report these abbreviations will be used:
S = styrene; SO = styrene-7,8-oxide

The Area Under the concentration-time Curve of SO in blood of rats and mice (AUC_{SO}) is considered to be a surrogate of the effective dose of SO. Based on this different scenarios of exposure to S or SO were analyzed.

From these results it appears that systematically available SO acts not as a potent carcinogen.

2. INTRODUCTION

Styrene (S) is an important chemical used in the production of polymers, copolymers and reinforced plastics. Exposure mainly occurs via inhalation in industries and operations producing or using styrene. A full description of the toxicology of styrene is given in several reviews: WHO (1983); BUA report on styrene (1989); MAK documentation for (1987). Information out of this literature is used in the following text without specific references.

Various studies have shown that the uptake of S is rapid and that it is distributed in the body via systemic circulation. S is mainly metabolized to SO and subsequently to various side chain oxidation products (e.g. mandelic acid, phenylglyoxylic acid). Concern about the carcinogenic potential of S has been related to the occurrance of SO as an intermediate metabolite of S. However, the results of controlled laboratory studies with animals (up to now 11 long-term studies) and various epidemiology studies have not provided clear evidence for the carcinogenicity of S.

As SO is formed from inhaled S in the body, explanations for the above findings could be:

- the epoxide is very efficiently detoxified resulting in extremely low systemic concentrations in the organism

- the epoxide is much less biologically active than other aliphatic epoxides or arene oxides

The kinetics of S and SO is important to describe quantitatively the fate of these substances in the exposed organism.

During the exposure to styrene (S) via inhalation the concentration of styrene-7,8-oxide (SO) in the organism is determined by the following factors:

- concentration of S in the atmosphere
- inhalation rate of S
- exhalation rate of S
- metabolic rate of S to SO
- rate of conversion of SO to other side chain oxidation products

The uptake of S via inhalation depends on the ventilation rate, the cardiac output, and the blood/air partition coefficient.

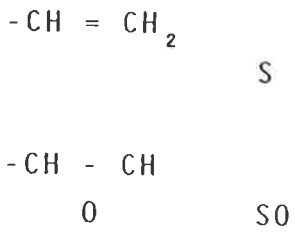
The metabolic rate of S depends upon site of metabolism, rate of blood flow to that site, the tissue/blood partition coefficient, substrate concentration as well as kinetic characteristics of the metabolizing enzymes. Similar considerations apply to the further conversion of SO.

At high substrate concentrations enzymatic processes always exhibit saturation kinetics. At low concentrations a metabolic rate often is limited by transport to metabolizing enzymes, with blood flow or ventilation rate as possible rate-determining steps and not by enzymatic capacity. Therefore, different routes of exposure to S or SO can lead to very dissimilar blood concentrations of SO, resulting in divergent biological effects.

A quantitative description of these relationships can be obtained by pharmacokinetic investigations using a closed chamber system. Accordingly in this study experiments have been performed with rats and mice under different conditions of exposure (to S and SO) to allow the prediction of blood concentrations of SO. These have been subsequently verified in additional experiments.

3. MATERIAL AND METHODS

3.1. Test Articles

Name:	Styrene (S) Styrene-7,8-oxide (SO)
Chemical Structures:	
Molecular Formulas:	S: C_8H_8 SO: C_8H_7O
Origin:	Aldrich
Batch Lot No.:	see raw data
Purity:	S > 99%; SO > 98%
Aggregate State:	S: liquid SO: liquid
Stability:	stable in inorganic solvents for at least 4 weeks
Storage:	-20°C in the dark

Preparations of test articles:	Undiluted S without further parification was used for inhalation experiments. SO was either used undiluted or for in vivo experiments dissolved in corn oil.

3.2. Test System

Animals:	1) Rats 2) Mice
Strain:	1) Sprague-Dawley 2) B6C3F1; NMRI*
Origin:	GSF, Neuherberg
Sex:	male
Weight:	Rats: about 160 - 300 g; Mice: 20 - 30 g checked immediately before dosing

* NMRI mice: origin GSF Neuherberg; due to limited supply of B6C3F1 mice these animals were used for experiments to measure tissue/blood partition coefficients; all other experiments were done with B6C3F1 mice.

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

Husbandry:

Room:	Animals were held in a climate controlled area of Kleintierhaus at GSF
Caging:	Acclimatization in macrolon cages, type 3
	Exposure in an all-glass inhalation system
Identification:	Individual number (tail marking)
Diet:	Pellet standard diet (Altromin, Lage)
Water:	Tap water ad libitum

3.3. Study Design

For clarification details on the study design for the individual experiments are described in the appropriate result sections.

3.4. Measurement and Calculations

3.4.1. Determination of S or SO in the gas phase

Gas samples were drawn by means of disposable syringes (1 syringe/sample) and immediately injected via injection loops of 5 ml in a Shimadzu GC-8-A or a Packard 437 A gas chromatograph. The gas chromatographs were equiped with stainless steel columns (2.5 m × 1/8 inch or 0.6 m × 1/8 inch) and flame ionization detectors (FID). The columns were packed with Tenax GC 60-80 mesh. Separation was accomplished isothermally at temperatures of 220°C (oven) and detection at 270°C (FID). Flow of nitrogen, hydrogen, and synthetic air was 60 ml/min, 60 ml/min, and 600 ml, respectively (Shimadzu GC-8-A), and 75 ml/min, 25 ml/min and 250 ml/min (Packard 437 A), respectively. Retention times for S were 1.1 min (Shimadzu GC-8-A) and 0.45 min (Packard 437 A).

For the determination of the enrichment of SO from air into liquids SO was also measured in the gas phase. Gas chromatographic conditions were the same as described for S, using the Shimadzu GC-8-A gas chromatograph, except the temperature of the oven was 230°C and that of the FID was 290°C. Retention time for SO was 3.7 min.

Peak areas were determined using an integrator 4290 (Varian). External calibration was performed by preparing different vapor concentrations of S or SO in desiccators of 6.4 and 20 l.

Linear regression analysis revealed correlation coefficients > 0.996 between peak areas and respective vapor concentrations.

The following equation was used to calculate the "liquid" volume of S or SO needed to produce a given concentration of S or SO in ppm in the atmosphere of the chamber.

$$V_l = \frac{MV_l \; V_D}{MV_g \; 10^6} \times X$$

V_l: liquid volume of S or SO (ml)
MV_l: mole volume of liquid S or SO (ml)
V_D: volume of the dessicator (ml)
MV_g: mole volume of an ideal gas at 25°C = 25130 (ml)
X: vapor concentration (ppm)

3.4.2. Determination of SO in blood samples

SO was directly determined in blood. The development of an analytical method which included extraction by n-hexane and determination by capillary gas chromatography with FID was developed in the course of another study; Kessler et al. (1990).

In certain experiments SO was measured in the n-hexane extract applying a second method developed in this study. Here a gas chromatograph HP 5890 II equipped with a mass selective detector (MSD) HP 5970 and an automatic injector HP 7673 (Hewlett Packard) were used. One µl of n-hexane extracts was injected on-column into a deactivated retention gap (0.53 mm, 2.5 m, Amchro) connected with a "MICONN"-connector (IfC, Bad Dürkheim) to a fused silica capillary HP 1 (0.2 mm, 10 m, Hewlett Packard). Helium was used as carrier gas with a flow rate of 0.8 ml/min at 35°C. The temperature of the column oven was held at 35°C for 0.25 min during injection and was then heated to 220°C with 30°C/min. Retention time of SO was 3.1 min. The temperature of the MSD transfer line was held at 250°C. Electron impact ionization of 70 eV was used. SO was selectively detected in the single ion mode by the fragment ion at m/z 89 using a dwell time of 100 ms. The electron multiplier voltage was set to 2000 or 2200 V. An external calibration curve of SO was prepared from 10 ng - 500 ng/ml SO in n-hexane. Linear regression analysis revealed correlation coefficients > 0.997 between peak areas and respective SO concentrations.

3.4.3. Calculation and statistics

- Calculation of the "Area Under the Curve" (AUC)

The area under experimental concentration-time curves was calculated by the trapezoidal rule. Where suitable, two or three e-functions were fitted to the experimental data and the area was calculated by integrating these functions.

- Statistics

Statistical calculations were carried out using the Student's t-test.

- Calculations for kinetics

Kinetic equations and calculations are described in detail in Filser (1991) on the basis of the model described there, concentration-time curves were calculated with a personal computer and fitted through the measured values using the software package "Kurven-Plotter" which has been developed specifically for this laboratory (Walter, 1987).

- Calculation for AUC_{SO}

The obtained relation between the external exposure to S, the metabolic rate of S and the resulting concentration of SO in blood can be used to calculate the AUC_{SO} in blood (ng x h/ml) for each S exposure. At first, the rates of S metabolism from exposure to S have to be calculated in dependence of time using the pharmacokinetic parameters obtained by means of the two-compartment model. Then, SO concentrations in blood corresponding to the diverse rates of S metabolism have to be taken out from Figs. 31 and 32 which are constructed on the basis of experimental data. Plotting the read values versus - sufficient long - time and integrating the obtained concentration-time curve for SO gives the dose surrogate AUC_{SO}.

3.4.4. Determination of partition coefficients

- Partition coefficients "liquid/air" ($P_{L/A}$) of S

Partition coefficients were determined at 37 °C using a headspace method (Hallier et al. 1981). A certain vapor concentration of S was produced in the gas phase of a closed system (desiccator of 6.4 l) by injecting the S as liquid. Prewarmed samples of water (100 ml), 0.25 M TRIS-HCl buffer pH 7.4 (100 ml) or olive oil (20 ml) were then introduced to the system and the concentration decline of S was measured in the gas phase by gas chromatography until the concentration in the exposure system remained constant. The partition coefficients were calculated using the following equation:

$$P_{L/A} = \frac{V_1 \, (Y_{1t=0} - Y_{1t->\infty})}{V_2 \, Y_{1t->\infty}}$$

$Y_{1t=0}$: concentration in the gas phase at t = 0 (ppm)
$Y_{1t->\infty}$: concentration in the gas phase at t -> ∞ (ppm)
V_1: volume of the gas phase of the closed system (ml)
V_2: volume of the liquid or organ (ml)

- Partition coefficients tissue/air ($P_{T/A}$) of S

Tissue sampling:

Human blood from healthy volunteers (male, female; age: 25 - 45) was collected from healthy volunteers by venipuncture using NH_4-heparinate-covered "Monovetten".

Samples from mice or rats were taken from animals following sacrifice with CO_2. Blood was collected from the abdominal vein into a NH_4-heparinate-covered tube. Liver, kidneys, lung, brain, selected muscle from the legs, whole gastrointestinal tract and subcutaneous fat were removed and homogenized immediately. Liver and kidney were homogenized with a glass potter, homogeniser and the other organs by means of an Ultraturrax. The homogenates were frozen in liquid nitrogen and then stored at -20°C for a maximum of one week before use. Samples from 4 -5 animals were pooled prior to the work up procedures.

Incubation:

Experiments were performed at 37°C in headspace vials (25 ml). 100 - 300 mg homogenate or 100 µl blood were transferred into the vials which were then closed by a teflon-covered septum. 2 ml of the vial gas phase were removed and replaced by 2 ml gas phase taken from a desiccator in which a certain vapor concentration of S had been prepared. The time course (1 - 8 h) of the concentration of S was determined in the gas phase of a set of vials. Each vial was measured once by analyzing 5 ml of the gas phase. The initial concentration was determined from a vial without any homogenate.

Additional experiments were performed with heated blood (60 °C, 15 min: preservation of the liquid state) and with boiled homogenates of liver and kidney (95 °C, 30 min) in order to inactivate metabolizing enzymes.

$P_{T/A}$ was calculated by the same way as $P_{L/A}$.

- Partition coefficients "liquid/air" ($P_{L/A}$) of SO

In principle, the method was the same as described above for S. Enrichment in 0.15 M K-phosphate buffer pH 7.4 and olive oil was determined using the desiccator system. However only 20 ml buffer or 5 ml of olive oil were used.

Determination of the SO enrichment in blood and water was the same as described for the enrichment of S in blood. However the liquid samples were added into the closed vial after the addition of the SO vapor. Due to the hydrolysis of SO in blood and water - or residual metabolism in blood - a constant "elimination process" occurs parallel to equilibration in the two phases. Therefore the concentration-time course of the measured values was analyzed by means of a two-compartment model assuming distribution between compartment 1 (gas phase) and compartment 2 (blood or water) and elimination from compartment 2 (Fig. 1A). $P_{L/A}$ was calculated with the following equation:

$$P_{L/A} = \frac{k_{12}\ V_1}{k_{21}\ V_2}$$

k_{12}: } microconstants of the transport between the phases
k_{21}: }

V_1 : compartment 1 = gas phase with volume 1
V_2 : compartment 2 = liquid phase with volume 2

The concentration of SO in the gas phase Y_1 is given by:

$$Y_1 = C_1 e^{\lambda_1 t} + C_2 e^{\lambda_2 t}.$$

The above constants were used to obtain k_{12}, k_{21} as described in Filser (1985).

$$k_{12} = - \frac{\lambda_1 C_1 + \lambda_2 C_2}{C_1 + C_2}$$

$$K_{21} = - (\lambda_1 + \lambda_2 + k_{12} + \frac{\lambda_1 \lambda_2}{k_{12}})$$

Y_1: concentration of SO in the gas phase

C_1, C_2: } intercept

λ_1, λ_2: } slope of functions

k_{12}, k_{21}: } microconstants of the transport between the phases

t: time

- Partition coefficients "tissue/blood" ($P_{T/B}$) of SO

Tissue sampling:

Rats and mice serving as donors for tissues and blood were pretreated i.p. with 600 mg/kg diethyl maleate (DEM) 15 min before sacrifice with CO_2 to reduce the tissue GSH level. Blood from rats was collected after decapitation in a beaker containing 0.5 ml heparin (Liquemin, Hoffmann-LaRoche, 1 : 10 with H_2O dest.). Blood from mice was collected via cardiac puncture using a disposable syringe, flushed with heparin. Subcutaneous fat (rat, mouse), sceletal muscles from the legs (rat, mouse), liver (rat) and esophagus (rat) were removed and boiled for 10 min (except fat). Blood and tissues were immediately used for experiments.

Incubation:

Prior to use pooled blood samples were incubated for 30 min at 37°C with 10 mM DEM, SO dissolved in methanol (1 mg/ml) was added to the samples giving a final concentration of 1 µg/ml blood.

$P_{T/B}$ of SO was determined at 37°C by incubating the respective tissue in the blood (pretreated as described above) of the respective species. Parallel incubations of SO were carried out in blood alone in order to determine residual metabolism. Incubations were performed in sealed reaction vials (2 ml, Eppendorf) containing 1 ml blood or 1 ml blood + 0.5 g organ, exceptions: + 0.1 g fat; + 0.124 g esophagus). The time course of the SO concentration in aliquots of 0.5 ml blood (one vial representing one time point) was measured by capillary gas chromatography with FID or alternatively with MSD (see

above). Because of the presence of a constant elimination process in blood, the concentration-time course of the measured values was analyzed by means of a two-compartment model assuming distribution between compartment 1 (blood) and compartment 2 (tissue) and elimination from compartment 1 (Fig. 1B). The partition coefficients were calculated with the following equation:

$$P_{T/B} = \frac{k_{12}\ V_1}{k_{21}\ V_2}.$$

k_{12}: } microconstants of the transport between the phases
k_{21}:

V_1: compartment 1 = volume of blood
V_2: compartment 2 = tissue

The concentration of SO in the blood of the blood + tissue incubate, Y_1, is given by:

$$Y_1 = C_1\ e^{\lambda_1 t} + C_2\ e^{\lambda_2 t}.$$

The above constants were used to obtain k_{12} and k_{21}:

$$k_{12} = -\ k_{el} - \frac{\lambda_1\ C_1 + \lambda_2\ C_2}{C_1 + C_2}$$

$$k_{21} = \frac{-C_1 C_2 (\lambda_1 - \lambda_2)^2}{(C_1 + C_2)[k_{el}(C_1 + C_2) + \lambda_1 C_1 + \lambda_2 C_2]}$$

Y_1: concentration of SO in blood

C_1: } intercept

C_2:

λ_1: } slope of functions

λ_2:

t:

k_{12}: } microconstants for the transport of SO between phases (matrices)

k_{21}:

The elimination rate constant k_{el} was determined from incubations with blood alone. Its concentration-time curve followed the e-function:

$$Y_1 = C\, e^{-k_{el} t}.$$

3.4.5. Reagents and solvents

All reagents and solvents used in these experiments were obtained from commercial suppliers and were reagent-grade or higher.

4. RESULTS

4.1. General remarks

For clarity a list of experiments performed in this study is shown below.

In order to show the relation to the study protocol and to provide some help for reading this section, in the following a list of performed experiments is given in analogy to the list of contents.

4.2. Partition homogenates of S and SO inbetween air, various liquids and tissues homogenates

- Partition coefficients "liquid/air" ($P_{L/A}$) of S
- Partition coefficients "tissue/air" ($P_{T/A}$) of S
- Partition coefficients "liquid/air" ($P_{L/A}$) of SO
- Partition coefficients "tissue/air" ($P_{T/A}$) of SO
- Partition coefficients "tissue/blood" ($P_{T/B}$) of SO

4.3. Inhalation kinetics of S in rat and mouse

- General remarks
- Inhalation without pretreatment (closed system)
- Inhalation without pretreatment (open system simulation)
- Inhalation after pretreatment with dithiocarb to inhibit metabolism
- Inhalation after pretreatment with S for one week

4.4. Kinetics of S after intraperitoneal administration to mouse or rat

4.5. Kinetics of S after oral administration to mouse or rat

4.6. Hydrolysis of SO at different pH values

4.7. Determination of SO in blood of rat and mouse

4.7.1. SO in blood after intravenous administration

4.7.2. SO in blood after oral administration

4.7.3. SO in blood after intraperitoneal administration

4.7.4. SO in blood during inhalative exposure against S

4.2. Partition of S and SO in between air, various liquids and tissue homogenates

The "liquid/air" and "tissue/air" partition coefficients of S in water, TRIS-HCl-buffer (pH 7.4), olive oil, in human blood, and various tissues of rats and mice are shown in Tab. 1. The enrichment of S in the various tissue was similar in rats and mice. S accumulates preferentially in the fat.

The "liquid/air" and "tissues/blood" partition coefficients of SO in water, TRIS-HCl-buffer (pH 7.4), olive oil, in rat blood, and tissues of rats and mice are summarized in Tab. 2. The constants of the e-functions used to calculate "tissue/blood" the partition coefficients are listed in Tabs. 21 - 26.

SO showed high enrichments from air into liquids. The "tissue/blood" partition coefficients for SO were similar for rats and mice. There was a slight enrichment in esophagus and liver, this effect was greater in fat. The value of 6 for "fat/blood" is also obtained by the ratio "olive oil/air"; "blood/air".

The data demonstrate that S enriches in olive oil or fat to a much greater extent than SO.

An example of the concentration time curve for SO in blood when incubated with "blood alone" and "blood + fat" is shown in Fig. 2. Instances when the AUC for SO in the blood was lower in incubations containing the tissue versus "blood alone" suggests that SO was eliminated within the tissue.

4.3. Inhalation kinetics of S in rat and mouse

- General remarks

Inhalation kinetics for S were measured using the "closed chamber technique" (CCT) which is described in detail in Filser (1992). During exposure to gaseous S animals (2 rats or 5 mice) were housed in a desiccator system (6.4 l). Exhaled CO_2 was trapped by soda lime and the atmosphere was replenished with an equivalent amount of O_2. Exposure was initiated following the administration of a predetermined amount of liquid S into the gas phase. To accelerate evaporation at higher concentrations, the liquid was applied to a hot ceramic boat. The decrease of the S concentration in the gas phase of the chamber was measured by gas chromatography. The rate of change in the concentration-time course was analyzed using a two-compartment model (Fig. 3). Compartment 1 represents the gas phase of the exposure chamber and compartment 2 the animal(s).

Pulmonary uptake and elimination are assumed to be first order processes. Thus the rate constants for inhalation (k_{12}) and elimination (k_{21}) are directly proportional to the actual concentration in the atmosphere and the actual average concentration in the organism, respectively. Metabolic elimination (K_{el^*}), is described as a Michaelis-Menten process where kel^* depends on V_{max} and an apparent Km_{app}.

- Inhalation kinetics without pretreatment (closed system)

The data obtained in inhalation studies with rats and mice are shown in Tab. 27 (rats) and Tab. 28 (mice). They are plotted semilogarithmically in Figs. 4 and 5. From the shape of the concentration-time curves follows that metabolism of S is saturable (Filser 1992). The solid lines in these figures represent the best fits, which than allowed the determination of the kinetic constants. Then, with these constants a conversion for an open system with an infinitely large atmospheric compartment was carried out. The kinetic parameters obtained for steady-state conditions are given in Tab. 3.

- In both species, similar values were determined for the thermodynamic partition coefficients.

- In rats, the bioaccumulation factor is lowest (2.7) and remained constant at concentrations below 10 ppm, in mice below 20 ppm (5.8).

- In both species, at concentrations below 10 ppm (rats) and 20 ppm (mice), inhaled S is metabolized almost quantitatively. This can be seen by comparing the values of the clearance of inhalation with those of exhalation and of metabolism, respectively. Similarly the ratios of metabolized and of exhaled S, respectively, to the amounts absorbed show the same relationship. The pulmonary retention is similar in both species. The half-life following a single exposure to S is very short (7 - 8 min) due to the fast metabolic elimination at the low concentration range.

Collectively these data show that at low exposure concentrations elimination of S via exhalation is neglegible.

× With respect to the individual animals the determined value for V_{max} was approximately 4 times higher in rats than in mice (Tab. 11). However, V_{max}/kg (accounting for differences in body weight) is 3 times higher in mice than in rats. The corresponding atmospheric concentration of S at which $V_{max}/2$ was reached between (190 ppm (rats), 270 ppm (mice), Tab. 3, Fig. 6).

These results show clearly that the mouse metabolizes S faster than rats.

The computed relation between the bioaccumulation factor of S in rats and mice at steady state versus atmospheric concentration is shown in Fig. 7. In both species, the bioaccumulation factor increases sharply at concentrations above about 300 ppm. This effect is due to the saturable metabolism of S. At very high concentrations the bioaccumulation factors approach the thermodynamic partition coefficients for S in rats and mice. In Fig. 8 the average steady-state concentrations of S in rats and mice are plotted against the atmospheric S concentration. The curves indicate that above 300 ppm the body burden of S rises sharply.

- Inhalation kinetics without pretreatment (open system simulation)

The data for S derived from closed chamber experiments were used to predict kinetic parameters of an open system (Tab. 4; Figs. 6 - 8).

Therefore further experiments simulating an open system were carried out to verify the predicted rates of metabolism of S. An open system is characterized by constant exposure concentration. To simulate such exposure conditions, S concentrations in the closed chamber were maintained approximately constant by repeated administration of S from a storage vessel into the atmosphere of the closed chamber containing either 2 rats or 5 mice. Thus the animals were exposed 8 h to mean S concentrations of 20 and 180 ppm (rats) and of 21 and 520 ppm (mice). The amounts of S needed to maintain "constant" concentrations were recorded. During the first 4 hours of exposure, S was not only metabolized, but also accumulated to the steady state equilibrium in the animals. This is called the "enrichment phase", similar to the first phase observed after administration of high initial S concentrations in the closed chamber experiments (Figs. 4 and 5) or after inhibition of the metabolism with dithiocarb (see below). During this phase S is taken up from the atmosphere faster than it is metabolized at the final steady state. Under steady state conditions, the amount of S necessary to maintain a constant atmospheric concentration is directly proportional to the amount metabolized during the observed time period. Consequently, only the amount of S administered during the second half of exposure period (hour 5 - 8) is considered to be metabolized at steady state conditions within this time period. Data from these experiments confirm the original pharmacokinetic analysis. The results are presented in Tab. 4, together with those predicted from the closed chamber experiments.

The general good agreement between the predicted and measured values demonstrates that the kinetic behavior of S in the open system can be predicted from the kinetic parameters derived from the closed system.

- Inhalation kinetics after single pretreatment with dithiocarb to inhibit S metabolism

Sodium diethyldithiocarbamate (dithiocarb) an inhibitor of monooxygenase-dependent metabolism (....) was administered i.p. in a saline solution (50 mg/ml) 10 to 15 min prior to the exposure to S. Rats received a dose of 200 mg/kg, mice a dose of 400 m/kg dithiocarb, respectively.

The concentrations of S measured in the closed chamber system during the exposure periods are given in Tabs. 29 and 30 and are plotted in Figs. 9 and 10 together with curves fitted using the two-compartment analysis (see above). For comparison, these figures contain also concentration-time curves and calculated curves from Figs. 4 and 5. From these data, it can be seen that dithiocarb is an effective inhibitor of S metabolism. After the initial uptake phase (ca. 4 hours) which represents mainly the enrichment of S in the animals, the further decline of the concentration is small when compared with that obtained with non-pretreated animals. Consequently, the loss of atmospheric S at exposure periods longer than 4 hours in the experiments with non-pretreated animals is assumed to be due to metabolism of S.

- Inhalation kinetics after pretreatment with S for one week

The potency of S to induce its own metabolism was investigated by exposing mice and rats over a period of 5 days (6 h/d) to S vapor concentration of 150 or 500 ppm. During this period the animals were housed in an open exposure system (240 l) supplied with charcoal-filtered and climate controlled air (25°C, 55% moisture). During the exposure period S was administered continuously into the chamber inlet duct. The concentration of the S vapor was measured by gas chromatography at regular intervals (0.5 to 1 h) at the inlet and the outlet of the chamber.

On the day following the 5th open chamber exposure the animals were exposed to S in closed chambers. Initial S concentrations were between 3200 and 5200 ppm. The atmospheric concentration-time course of S was followed (see Tabs. 31 - 34 and Figs. 11 - 14). These values were plotted. The figures also contain predicted curves produced using the assumption that no induction of the metabolism of S would occur. (Data derived from Fig. 4 + 5.) From these results we conclude that repeated exposure to S did not change the rate of metabolism of S. These findings are in agreement with those recently published by Mandrela et al. (1992).

4.4. Kinetics of S after intraperitoneal administration of S

S was administered i.p. as undiluted liquid using of a microliter syringe. Rats received 5, 10, or 50 µl per animal (about 20, 40 or 200 mg/kg), mice received 5 or 10 µl per animal (about 160 or 320 mg/kg). Immediately after administration, one rat or two mice were placed into the closed chamber inhalation system (6.4 l) and the concentration-time course of the exhaled S was followed in the gas phase of the system. The results are shown in Tabs. 35 and 36 and in Figs. 15 - 17. These figures also contain curves predicted from the inhalation studies by means of a two-compartment model. The model considers the animal to be a single homogenious compartment only in which the administered S is distributed instantaneously. Such an ideal distribution cannot be assumed immediately after the administration of lower doses of S. Therefore it is understandable that in the experiments more S is found to be exhaled during this period than predicted.

Except for the above mentioned initial deviation the measured data from rats and the corresponding AUC values (Tab. 5) do agree reasonably good with the predicted curves. From these results it is concluded that within the range of administered i.p. doses no first-pass effect occurred in rats.

Although the results from mice are qualitatively similar such firm conclusions cannot be drawn.

4.5. Exhalation kinetics of S after oral administration

S was administered orally as a solution in olive oil at dose levels of about 100 or 170 mg/kg (rats) and 170 or 350 mg/kg (mice, respectively. After dosing, one rat or two mice (dose: 170 mg/kg) were placed into the closed chamber system (capacity 6.4 l) or one mouse (dose: 350 mg/kg) was placed into the chamber (capacity 0.86 l). The concentration-time course of he exhaled S was followed in the gas phase of the system.

The results are shown in Tabs. 37 and 38 and in Figs. 18 - 21. These figures contain curves predicted from the inhalation studies by means of the two-compartment model assuming no first-pass effect. Although there is, due to the oral administration, a considerable variation of data between animals the results allow some qualitative conclusions. The shape of the curves resulting from measured values per animal is similar to the predicted one. Some are even in reasonable good agreement indicating that no first pass occurred. However, the large variation of data and corresponding AUC (see Tab. 6) values does not allow any further interpretation for both species.

4.6. Hydrolysis of SO at different pH values

The rates of the hydrolysis of SO at 37 °C as a function of pH were determined. SO dissolved in methanol (10 mg/ml) was added at 37 °C to culture tubes (10 ml) containing H_2O (pH 6.2.), different concentrations of HCl in H_2O (pH 3 and 4) or 0.2 M potassium phosphate buffer in H_2O (pH 6 and 7). The final concentration of SO was 3 and 6 µg/ml (H_2O pH 6.2.) and 100 µg/ml (HCl, buffer). At certain time points following the addition of SO, the reaction was terminated by the extraction of unreacted SO with n-hexane. The concentration of SO in the extracts was than measured.

Results are presented in Tab. 20 and in Figs. 25 and 26. Fig. 25 shows the concentration-time curves obtained at the different pH values. The corresponding curves (solid lines) were calculated by linear regression analysis. From these e-functions half-lifes were computed and are given in the figure. Fig. 26 shows the relationship between half-lifes of SO and pH. The solid line gives the regression curve obtained in non-buffered aqueous systems. The broken line is fitted by eye through the buffered systems. From that figure it is apparent that half-life of SO is strongly dependent on the pH and that there is a significant buffer effect. Similar results on the hydrolysis reactions of SO have already been published by Ross et al. (1982). Based on the short half-life time of SO at low pH values, it can be expected that only relatively small amounts or orally administered SO may reach the liver and the systemic circulation.

4.7. Determination of SO in blood of rat and mouse

General remarks:

Non radioactive SO was used in these experiments; the analytical method of Kessler et al. (1990) was applied.

4.7.1. SO in rat blood after intravenous administration of SO

Undiluted SO was administered (tail vein injection) at the dose level of 25 mg/kg body weight using a microliter syringe with a dosing cannula. At selected time intervals single animals were sacrifice (CO_2 as phyxation) and the SO concentration in blood was measured.

Results are presented in Tab. 8 and in Fig. 22. The AUC_{SO} of the concentration-time curve is shown in Tab. 9. Blood concentration of SO diminished rapidly during the first minutes following dosing. This could be interpreted to be due to distribution within the whole body. However, after about 5 min the slope of the curve resembled that calculated for i.p. administration to rats.

4.7.2. SO in blood after oral administration of SO

SO was administered orally to rats (200 mg/kg body weight) and mice (200 and 500 mg/kg body weight) in olive oil. At selected time points after administration single animals were sacrificed and the SO concentration in blood was measured.

Experimental data are presented in Tabs. 10 and 11 and in Figs. 23 and 24. $AUC_{SO's}$ of the concentration-time curves are given in Tabs. 12 and 13.

In both species large interanimal variation of blood plasma levels was found. These occured at all dose levels examined. Despite this variation the bioavailability of SO in the blood after oral administration was found to be < 5% of the values obtained after i.p. administration of the same doses.

The concentration-time curves after oral administration of 200 mg/kg (rat) and 500 mg/kg (mouse) show a decrease at 30 min. This phenomenon has also been described by others (Kim et al. 1990). This is interpreted to be an effect of the dosing vehicle (olive oil) which may lead to a biphasic absorption from the gastrointestinol tract.

4.7.3. SO in blood after intraperitoneal administration of SO

Undiluted SO was administered i.p. to rats (200 mg/kg body weight) and mice (100; 200; 500 mg/kg body weight). At selected time intervals single animals were sacrificed and the SO concentration in blood was measured.

Experimental data are presented in Tabs. 14 and 15 and in Figs. 23 and 27. AUC_{SO} values from the concentration-time curves are given in Tabs. 16 - 17. In rats, the AUC_{SO} after 200 mg/kg is about 1.5 times greater than in mice. This is interpreted that mice may metabolize SO 1.5 times faster than rats.

4.7.4. SO in blood during exposure to S

SO was determined in whole blood of rats and mice immediately and up to 3 hours after exposure to S vapor under steady-state conditions. The steady state can either be reached by continuous inhalation of a constant

atmosphere or with an exposure start at high concentrations (bolus level) followed by a period of continuous decrease to the desired level due to metabolism and excretion of S by the animal in the chamber. From the inhalation kinetics (see section 4.3.) it can be concluded that following a start at high concentrations equilibrium in a closed chamber (20 l for 3 - 4 rats and 6.4 l for 3 - 4 mice) is really reached after 3 to 4 hours if the start concentrations are in appropriate ratios to the required steady state level, e.g. for rats:

steady state level	start level	
20	200	
260	1,460	
350	2,300	
510	3,400	} limit due to vapor pressure
800	3,400	

After reaching the required level the steady state was maintained for at least 3 hours by repeated injections of S (as liquid or as vapor, depending on the concentration) into the atmosphere of the chamber. At the end of the exposure all animals were removed. Animals were sacrificed at hourly intervals from the termination of the exposure. Afterwards the SO concentration in blood was measured.

The results are shown in Tabs. 18 and 19 and in Figs. 28 and 29 together with the calculated linear regression lines.* Regression analysis served to determine the respective half-lifes, which are also presented in the figures. In rats, the half-life times at every exposure concentration of S are longer than in mice. It is certainly an important observation that even 2 hours after

Note: The results show a considerable variation between single data points. However, it should be kept in mind that each value at the various time points results from a different animal which exert the usual interindividual variation in their kinetics of xenobiotics.

the exposure to 20 ppm of S (the current MAK value in Germany), SO could be found in the blood of rats. In several control experiments using fresh blood of rats not exposed to S or SO, no SO was detectable (see "BASF Project No.: 21B0705/879028"). Fig. 30 shows a plot of the blood concentration of SO at the end of exposure to S versus the corresponding atmospheric concentrations of S.

Below the exposure concentration of 260 ppm S the blood level of SO (about 200 ng/ml) was similar in rats and mice. Above this exposure concentration blood levels of SO reached their peak in rat blood at about 400 ng/ml. By contrast the SO concentration in mouse blood increased to about 6000 ng/ml without evidence of a peak being reached. In Figs. 31 and 32 the concentration of SO determined in the blood of animals at the end of exposures is plotted against the respective metabolic rates of S, calculated pharmacokinetically. For rats, a linear correlation was found indicating that at the end of exposure blood SO concentration was directly dependent on the metabolic rate of S. For mice, however, blood SO concentration at the end of exposure did not depend solely on the metabolic rate of S, but it appeared also to depend on the rate at which SO was eliminated.

Using methods described in section 3.4.5. AUC_{SO} in blood of rats was estimated for exposures to 1000 ppm S (6 h) and to 20 ppm S (8 h). The obtained values were:

- AUC_{SO} (1000 ppm S) = 4400 [ng x h/ml]
- AUC_{SO} (20 ppm S) = 140 [ng x h/ml]

5. DISCUSSION

- Enrichment of S and SO in liquids and tissues

Both, S and SO, accumulate preferentially in lipid tissues. S accumulates almost exclusively in lipids as it becomes evident from the ratio of the partition coefficients "olive oil/air" to "TRIS-HCl-buffer (pH 7.4)/air" which was 400. For SO however, the ratio of the corresponding coefficients was determined to be 15, indicating that the hydrophilic properties of SO are much more pronounced.

Partition coefficients "fat/blood" of S in rats and mice are estimated to be between 41 and 44, respectively. The corresponding figures for SO were 4.6 and 6.1 (mice and rats). The findings suggest that SO, in contrast to S, is distributed within the organism almost homogeneously.

- Gas uptake kinetics of S in rat and mouse

  - Inhalation

The rate of metabolism of S is concentration-dependent in both species. At steady state, it increased almost linearily with the atmospheric exposure concentration up to about 300 ppm. Below this concentration accumulation of inhaled S is limited. The bioaccumulation factor at steady state, a measure of the enrichment, increased from 2.7 below 10 ppm to 13 at 300 ppm in rats and from 5.8 below 20 ppm to 13 at 300 ppm in mice. These small bioaccumulation values at low exposure concentrations (around 20 ppm) result from effective metabolic elimination. At these concentra-

tions, transport to the metabolizing enzymes and not their metabolic capacity is the rate limiting step for metabolism ("transport limitation"). Kinetic behaviour of S is strongly influenced by physiological parameters as blood flow and especially the alveolar ventilation rate. Therefore, in both species similar results were obtained with respect to the pulmonary retention and the ratios of metabolized S and of exha2ed S to inhaled S. Thus, the rate of metabolism at steady state increased linearily with the atmospheric exposure concentration of S to about 300 ppm. For the same reason it can be expected, that in this exposure range the rate of metabolism should not be influenced by xenobiotics capable of inducing the oxidative metabolism of S. This conclusion is supported by the in vivo findings where in both species no change in the rate of S metabolism occurred under the conditions described for these experiments. This is in agreement with data from Nolan (1991), however, investigators using different exposure conditions (500 ppm/24 h) have reported about the induction (E. Elovara et al., 1991).

At higher concentrations of S in the atmosphere, the rate of its metabolism at steady state is progressively limited by biochemical parameters of the metabolizing enzymes (V_{max} and Km_{app}). At about 600 ppm (rats) and 900 ppm (mice) V_{max} of 224 µmol/(h × kg) (rats) and of 625 µmol/(h × kg) (mice) is reached. Consequently, S accumulated at these levels in the fatty tissue to a much higher extend than at low concentrations. Above 300 ppm, the bioaccumulation factor "organism/air" increased rapidly with increasing concentration; at concentra-

tions above 2000 ppm S, it finally reached its maximum (410 - 420). This is equivalent to the thermodynamic partition coefficient "organism/air" of S.

- Kinetics of S after intraperitoneal and oral administration

In several long-term bioassays S was administered orally to rats and mice and intraperitoneally to rats. To study bioavailability of S following these types of treatment, kinetics of S following intraperitoneal and oral administration was investigated in both species and compared with inhalation kinetics of S. In rats, the measured concentration-time curves of intraperitoneally administered liquid S could be predicted accurately using the kinetic parameters obtained from the inhalation experiments. Obviously, intraperitoneally administered S is taken up rapidly into the blood stream. No first-pass effect was observed in the rat at administered doses up to 240 mg/kg. In mice, the curves observed after administration of S up to 322 mg/kg were below the predicted ones. This difference might be caused by a slower bioavailability of intraperitoneally administered S and by a first pass effect in the liver.

Concentration-time curves following oral administration to mice and rats were not predictable. Here, kinetic relations are complex. Already the uptake into blood is highly influenced by the amount and the type of dose vehicle used (Kim et al., 1990).

- Kinetics of SO in blood of rat and mouse

According to Haber's rule, the areas under the concentration-time curves of SO in blood (AUC_{SO}) can be regarded as a dose surrogate for SO. Using kinetic data described below the AUC_{SO} can be calculated for different exposure routes for both species (Filser, 1992). Pharmacokinetic measurements of SO from blood following exposure to atmospheric S or to intraperitoneal, intravenous or oral administration of SO were performed after developing a sensitive direct assay (Kessler et al., 1990).

- SO in blood during exposure against S

SO was found in blood of both species following exposure under steady-state conditions to atmospheric concentration of S between 20 and 800 ppm. In rats, blood concentrations of SO were linearly correlated with the rate of metabolism of S. This indicates that in this species at all S concentrations tested the rate-limiting step is the formation of SO rather than its detoxification. In mice blood concentrations of SO following exposure to S concentrations below 260 ppm were similar to those in rats. In contrast to rats, a sharp increase of SO levels was found at higher S concentrations. This is interpreted as follows: At lower concentrations of S, the relatively higher metabolism of S to SO in mice (see above) is compensated by a greater elimination rate of the formed SO. This leads to comparable body burden of SO in both species. At higher S concentraitons, greater amounts of SO become systematically available in mice. This may be due to a

depletion of glutathione consumed by the reaction with the intrahepatically formed SO. Whereas in rats blood concentrations of SO did not exceed 460 ng/ml, in mice levels up to 7500 ng/ml were reached during exposure to 800 ppm S. Using the above described results the AUC_{SO} for both species can be calculated (Filser, 1992).

- SO in blood after intraperitoneal and intravenous administration

In mice, the ratio of AUC_{SO}/dose increases with increasing doses (i.p. 100, 200, 500 mg). This might be indicate saturability of the SO pathway. In rats at 200 mg/kg, the ratio was about 1.5 times higher than in mice. This confirms the findings from the inhalation experiments with S, where metabolism of SO was found to be faster in mice (see above).

From SO blood concentrations measured after intraperitoneal administrattion of 200 mg/kg SO to rats an AUC_{SO} can be determined experimentally. For the same treatment regimen an AUC_{SO} can be predicted based on inhalation data. The comparison of these two AUC values shows that the predicted AUC value (21.2 mmol × h/ml) is 7 times lower than the determined AUC value. This difference may result from the saturation of metabolism of i.p. administered SO (see Nolan, 1991).

In addition, the difference could also indicate an intracellular first pass metabolism of SO formed metabolically "in situ" resulting in effective elimination and low systemic availability of this metabolite. Similar findings have been reported with 1,3-butadiene and ethylene (Filser et al., 1984).

After intravenous SO administration to rats (25 mg/kg), the ratio of the AUC_{SO} to the amount administered was about 3 times higher than the corresponding ratio obtained after intraperitoneal administration of SO (200 mg/kg). This indicates a first-pass effect by the liver for intraperitoneally administered SO even at 8 times higher doses.

- SO in blood after oral administration of SO

Following administration of 200 mg/kg to rats and of 200 and 500 mg/kg to mice the concentration of SO in blood was determined. The results showed interanimal variation and several maxima in the curve. In mice, the AUC_{SO} at 200 mg/kg was 50 times lower than that observed after 500 mg/kg. This may be an effect of the dose vehicle.

The comparison of the AUC_{SO} after oral dosing with the analogue curve after i.p. dosing (description of experimental results see below) shows that the bioavailable amount of SO after oral administration is only 0.14 to 4% of the amount after i.p. administration. It is assumed that this difference is at least partly due to the fast hydrolysis of orally administered SO at the acidic pH in the stomach. At pH 7

(37 °C) the hydrolysis of SO has a half-life of several hours. The half-life decreased rapidly with pH to 0.4 min at pH 3.

The results of these experiments show also that detectable amounts of SO do reach the circulation. Under similar treatment conditions no systemic tumors were observed. This suggests a low tumorigenic potency for SO.

- Conclusion

Concern about the carcinogenic potency of S has been related to the occurrence of SO as an intermediate metabolite of S. The pharmacokinetic parameters for S and SO determined in this study permit quantitative description of major pathways of both S and SO in rat and mice under various conditions of exposure. For illustration the following examples of exposure scenarios are provided.

An estimation of AUC_{SO} for a 6-hour exposure of rats to S (1000 ppm) gives about 4400 ng × h/ml of blood. Following oral administration of SO (200 mg/kg) the AUC_{SO} of about 720 ng × h/ml blood is determined. For an inhalative exposure of rats to S at 20 ppm for 8 hours the AUC_{SO} can be estimated to be around 140 ng × h/ml of blood which is 4 times less than AUC_{SO} for oral dosing (see following table).

	Route/Dose/Concentration	AUC_{SO} in Blood	Ratio AUC_{SO}'s
SO	oral 200 mg/kg	717	-
S	inhalation 1000 ppm/6 h	4400	6
S	inhalation 20 ppm/8 h	140 (estimation)	0.2

In long-term studies with oral administration of SO to rats (50 and 250 mg/kg, once daily, 4 - 5 days/week, 52 weeks; Conti et al.; 1988) tumors of the forestomach, but no systemic tumors have been observed. Therefore, it seems that in rats systemically available SO does not act as a potent carcinogen. This reduces concerns of possible adverse effects of SO formed by metabolism upon exposure to styrene.

6. REFERENCES

Beratergremium für Umweltrelevante Altstoffe (BUA) der GDCh (ed.) (1990).
Styrol (Ethenylbenzol); Verlag Chemie, Weinheim

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

E. Elovaara, K. Engström, T. Nakajima, S.S. Park, H.V. Gelboin and H. Vainio (1991):
Metabolism of inhaled styrene in acetone, phenobarbital- and 3-methylcholanthrene-pretreated rats: stimulation and stereochemical effects by induction of cytochromes P45011E1, P45011B and P4501A.
Xenobiotica 21, 651 - 661

J.G. Filser (1992):
The closed chamber technique - uptake, endogenous production, excretion, steady-state kinetics and rates of metabolism of gases and vapors.
Arch. Toxicol. 66, 1 - 10

J.G. Filser (1985):
Bestimmung pharmakokinetischer Parameter flüchtiger Fremdstoffe im abgeschlossenen Expositionssystem unter Benutzung von n-Hexan als experimentelles Beispiel.
Johannes-Gutenberg-Universität, Mainz

J.G. Filser and H.M. Bolt (1984):
Inhalation pharmacokinetics based on gas uptake studies. VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats.
Arch. Toxicol. 55, 219 - 223

E. Hallier, J.G. Filser and H.M. Bolt (1981):
Inhalation pharmacokinetics based on gas uptake studies. II. Pharmacokinetics of acetone in rats.
Arch. Toxicol. 47, 293 - 304

D. Henschler (ed.) (1987):
Kommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe der DFG: Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 13. Lieferung, "Styrol".
Verlag Chemie, Weinheim

International Programme on Chemical Safety (IPCS) (ed) (1983):
Environmental Health Criteria 26, "Styrene". WHO, Geneva

W. Kessler, X. Jiang and J.G. Filser (1990):
Direct determination of styrene-7,8-oxide in blood by gas chromatography with flame ionization detection.
J. Chromatog. Biomed. Appl. 534, 67 - 75

H.J. Kim, J.V. Bruckner, C.E. Dallas and J.M. Gallo (1990):
Effect of dosing vehicles on the pharmacokinetics or orally administered carbon tetrachloride in rats.
Toxicol. Appl. Pharmacol. 102, 50 - 60

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

R.J. Nolan, P.W. Langvardt, D.A. Markham and F.A. Smith (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 SIRC

G. Walter (1987):
Pharmakokinetik von n-Hexan, Toluol und 2-Butanol.
Diplomarbeit, Ludwig-Maximillians-Universität, München

7. TABLES

TABLE 1

Section 4.2.

Partition coefficients "liquid/air" (PL/A) and "tissue/air" (PT/A) of styrene at 37°C

Liquid		PL/A x ± SD	n
water		15 ± 1.5	4
0.25 M TRIS-HCl-buffer, pH 7.4		14 ± 0.6	3
olive oil		5600 ± 58	3
Tissue	**Species**	**PT/A x ± SD**	**n**
blood	rat	110 ± 4.7	5
	mouse	100 ± 7.5	4
	man	48 ± 7.6 *	4
fat	rat	4500 ± 430	5
	mouse	4400 ± 160	4
brain	rat	140 ± 14	3
	mouse	124 ± 9.5	3
GI-tract	rat	180 ± 30	3
	mouse	180 ± 39	3
liver	rat	130 ± 24	3
	mouse	120 ± 11	3
kidney	rat	120 ± 18	4
	mouse	180 ± 15 *	3
muscle	rat	94 ± 17	4
	mouse	130 ± 17 *	3
lung	rat	70 ± 19	3
	mouse	70 ± 14	3

* = sign. different ($2p < 0.05$) compared to rat

TABLE 2

Section 4.2.

Partition coefficients "liquid/air" (PL/A) and "tissue/blood" (PT/B) of styrene-7,8-oxide at 37 °C

Liquid	PL/A x ± SD	n
water	528 ± 120	4
0.25 M TRIS-HCl-buffer pH 7.4	965, 896	2
olive oil	14000 ± 1670	3
rat blood	2370 ± 865	3

Tissue	Species	PT/B x ± SD	n	AUC_{T+B}/AUC_B x ± SD
fat	rat	6.1 ± 0.9	5	0.83 ± 0.06
	mouse	4.6 ± 0.69	3	0.77 ± 0.02
muscle	rat	1.5 ± 0.51	4	0.86 ± 0.08
	mouse	1.1 ± 0.18	3	0.74 ± 0.01
liver	rat	2.6 ± 0.83	3	0.71 ± 0.05
esophagus	rat	2.2 ± 0.35	3	0.99 ± 0.04

Single values are presented in Tables 21 - 26.

TABLE 3

Kinetic parameters for styrene

Styrene Kinetics

(open system; V_1 -> ∞; 1 rat and 1 mouse)

Parameter	Formula	Value		Dimension
		Rat	Mouse	
Thermodynamic partition coefficient (organism/air)	$\frac{V_1 k_{12}}{V_2 k_{21}}$	410	420	$\frac{\text{nl gas/ml tissue}}{\text{ppm in atmosphere}}$
Bioaccumulation factor at steady state (b) (organism/air)	$\frac{V_1 k_{12}}{V_2 (k_{21} + k_{el})}$	2.7	5.8	$\frac{\text{nl gas/ml tissue}}{\text{ppm in atmosphere}}$
Clearance of inhalation (a)	$V_1 k_{12}$	3,800	730	ml/h
Clearance of exhalation (a, b)	$\frac{V_1 k_{12} k_{21}}{k_{21} + k_{el}}$	25	10	ml/h
Clearance of metabolism (a, b)	$\frac{V_1 k_{12} k_{el}}{k_{21} + k_{el}}$	3,800	720	ml/h
Half-life (b)	$\frac{\ln 2}{k_{21} + k_{el}}$	0.12	0.13	h
$\frac{\text{Amount metabolized}}{\text{Amount absorbed}}$ (b)	$\frac{k_{el}}{k_{21} + k_{el}}$	0.99	0.99	-
$\frac{\text{Amound exhaled}}{\text{Amount absorbed}}$ (b)	$\frac{k_{21}}{k_{21} + k_{el}}$	0.0066	0.014	-
Pulmonary retention (b)	$\frac{\text{cl. of metab.}}{\text{ventil. rate}}$	48	54	%
V_{max}	-	56	15	µmol/h
V_{max}/kg	-	224	625	µmol/h
Km_{app}	-	1,000	3,000 - 5,000	nl gas/ml tissue
Atmospheric concentration at $V_{max}/2$	-	190	270	ppm

a: related to atmospheric concentration

b: below 10 ppm (rat), and 20 ppm (mouse), respectively

Pharmacokinetic parametrs of styrene are calculated for one rat (250 g) and one mouse (24 g), respectively. Ventilation rate was set to be 8 l/h (rat) and 1.3 l/h (mouse), respectively.

TABLE 4

Rate of metabolism experimentally determined and predicted using the two-compartment pharmacokinetic model

Styrene Kinetics

(open system; V_1 -> ∞; 2 rats and 5 mice)

Spec.	No. exp. [n]	V_2 [ml]	atm. conc. [ppm]	dN_{el}/dt measured [µmol/h] $\bar{x}$	dN_{el}/dt measured [µmol/l] range	dN_{el}/dt predicted [µmol/h]
Rat	3	400	180	42	33 - 50	50
	2	480	20	12	12 - 12	7
Mouse	2	130	520	73	70 - 75	70
	2	120	21	4.8	3.9 - 5.6	3

Used formulas and definition of variables see sections:

TABLE 5

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats or mice

Areas under the concentration-time curve in the gas phase

Species (animal/ exper.)	Number of Exper. [n]	$Y_2(0)$ [µmol/ml tissue]	$AUC_{meas.}$ [ppm*h] x ± SD	$AUC_{pred.}$	$\frac{AUC_{meas.}}{AUC_{pred.}}$
Rat (n=1)	2	0.21	8.3; 9.9	8.3	1.1
Rat (n=1)	3	0.39	39 ± 1.0	27	1.4
Rat (n=1)	3	2.0	380 ± 120	510	0.75
Mouse (n=2)	3	1.76	56 ± 13	96	0.58
Mouse (n=2)	2	3.2	140; 310	430	0.52

Used formulas and definition of variables see section: 4.4.

TABLE 6

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats and mice

Areas under the concentration-time curve in the gas phase

Species (animal/ exper.)	Number of Exper. [n]	$Y_2(0)$ [µmol/ml tissue]	$AUC_{meas.}$ [ppm*h] x ± SD	$AUC_{pred.}$	$\frac{AUC_{meas.}}{AUC_{pred.}}$
Rat (n=1)	5	0.96	47 ± 24	130	0.38
Rat (n=1)	3	1.7	170 ± 21	380	0.45
Mouse (n=2)	5	1.6	17 ± 9	120	0.15
Mouse (n=1)	2	3.4	15; 550	500	0.57

Single values are given in Tables 37 and 38.

TABLE 7

Styrene-7,8-oxide kinetics in blood after application to rats and mice.
Summary of AUC* values from tables 9, 12, 13, 16 and 17

Dose; Application	Species	AUC* calculated via e-functions [µg × h/ml]	AUC* calculated via traperoidal rule [µg × h/ml]
25 mg/kg, i.v.	rat	6,250	8,270
200 mg/kg, p.o.	rat	-	717
200 mg/kg, i.p.	rat	16,487	19,680
200 mg/kg, p.o.	mouse	16.4	11.2
500 mg/kg, p.o.	mouse	883	851
100 mg/kg, i.p.	mouse	2,708	1,416
200 mg/kg, i.p.	mouse	11,782	12,190
500 mg/kg, i.p.	mouse	67,443	58,000

* AUC* = Area Under the Concentration time curve

TABLE 8

Data for section 4.7.1.

Styrene-7,8-oxide in blood after intravenous application of styrene oxide to rats

25 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
1	158,000	231
2	13,200	204
3.5	1,230	229
5	4,120	206
10	1,890	210
20	1,050	270
30	81	270
45	766	268
60	53	232
90	544	274
120	5.5	231
150	53	237
180	1	230
240	1.5	238

TABLE 9

Data for section 4.7.1.

Styrene-7,8-oxide kinetics in blood after intravenous application; rat

25 mg/kg:

AUC in blood calculated by:	e-functions [$Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}$]	trapezoidal rule
C_1 [ng/ml]	500,000	
C_2 [ng/ml]	2,875	
λ_1 [h^{-1}]	-100	
λ_2 [h^{-1}]	-2.3	
AUC* [ng × h/ml]	6,250	8,270

*: Area under the curve

TABLE 10

Data for section 4.7.2.

Styrene-7,8-oxide in blood after oral application of styrene oxide to rats

200 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
5	3,561.00	222
5	40.08	230
5	74.16	216
15	126.24	221
15	3,614.88	268
30	16.44	233
30	109.44	227
30	16.68	221
30	12.24	256
30	18.72	221
60	74.16	269
60	19.96	218
60	418.10	265
60	331.08	257
90	303.40	217
90	88.20	265
90	5.16	239
90	2.88	235
120	11.40	244
120	9.60	267
120	3.50	237
150	7.79	223
150	3.42	222
150	3.96	242

TABLE 11

Data for section 4.7.2.

Styrene-7,8-oxide in blood after oral application of styrene oxide to mice

200 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
5	3.1	26
5	10.9	20
15	11.2	24
15	12.4	20
30	13.4	23
30	8.3	20
45	9.6	24
45	6.6	23
60	3.6	20
60	6.8	24
90	4.4	21
90	4.9	21

TABLE 11 (continued)

Data for section 4.7.2.

Styrene-7,8-oxide in blood after oral application of styrene oxide to mice

500 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
5	668	25
5	85	25
5	27	28
15	57	29
15	181	26
15	7,021	28
15	6,220	26
30	78	24
30	157	25
30	47	26
45	399	26
45	31	24
60	40	22
60	35	22
75	50	25
75	39	24
90	4.8	23
90	11	25
90	76	21
90	7.5	30
120	4.1	24
120	4.3	25
120	45	28
150	3.4	29
150	1.3	30
150	1.9	27

TABLE 12

Data for section 4.7.2.

Styrene-7,8-oxide kinetics in blood after oral application; rat

200 mg/kg

AUC in blood calculated by:	trapezoidal rule
AUC* [ng × h/ml]	717

*: Area under the curve

TABLE 13

Data for section 4.7.2.

Styrene-7,8-oxide kinetics in blood after oral application; mouse

200 mg/kg:

AUC in blood calculated by:	e-functions $[Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}]$	trapezoidal rule
C_1 [ng/ml]	-32	
C_2 [ng/ml]	15	
λ_1 [h^{-1}]	-18.5	
λ_2 [h^{-1}]	- 0.84	
AUC* [ng × h/ml]	16.4	11.2

*: Area under the curve

TABLE 13 (continued)

Data for section 4.7.2.

Styrene-7,8-oxide kinetics in blood after oral application; mouse

500 mg/kg:

AUC in blood calculated by:	e-functions [$Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t} + C_3 \times e^{\lambda_3 t}$]	trapezoidal rule
C_1 [ng/ml]	-150,000	
C_2 [ng/ml]	24,000	
C_3 [ng/ml]	200	
λ_1 [h^{-1}]	-25	
λ_2 [h^{-1}]	- 8.3	
λ_3 [h^{-1}]	- 1.5	
AUC* [ng × h/ml]	883	851

*: Area under the curve

TABLE 14

Data for section 4.7.3.

Styrene-7,8-oxide in blood after intraperitoneal application of styrene oxide to rats

200 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
3	1,691	218
3	18,900	244
5	21,696	230
5	30,624	224
15	12,008	226
15	32,396	220
15	59,112	256
45	2,595	220
45	6,204	242
45	20,628	236
60	552	240
60	3,072	224
60	5,010	242
90	318	230
90	480	210
90	336	226
120	69.6	220
120	38.4	218
120	45.6	226
180	25	234
180	3.6	232
180	11	236
210	2.62	246
210	2.86	236
210	3.2	226
240	6.68	228
240	5.88	226
240	5.3	226

TABLE 15

Data for section 4.7.3.

Styrene-7,8-oxide in blood after intraperitoneal application of styrene oxide to mice

100 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
5	5,356	25
15	(45)	(26)
15	1,897	23
30	(34)	(23)
30	3,859	25
45	106	25
60	62	24
75	9.2	23
90	4.8	25
120	3.8	24

Values in brackets were not taken into account.

TABLE 15 (continued)

Data for section 4.7.3.

Styrene-7,8-oxide in blood after intraperitoneal application of styrene oxide to mice

200 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
5	11,925	21
5	138	25
5	1,540	25
15	47,600	26
15	56,887	24
15	32,900	21
30	1,632	29
30	2,336	23
30	16,600	23
45	2,380	28
45	261	25
45	76	24
60	140	24
60	260	25
60	588	25
90	12	22
90	44	23
120	11	23
120	6.8	24
120	4.8	29
150	1	26
150	8	24
150	7.1	22
150	3.9	27

TABLE 15 (continued)

Data for section 4.7.3.

Styrene-7,8-oxide in blood after intraperitoneal application of styrene oxide to mice

500 mg/kg:

Time [min]	Conc. [ng SO/ml blood]	Body weight [g]
5	25,638	24
15	114,878	25
30	26,529	24
45	42,928	24
60	17,743	22
90	1,258	23
120	14.3	22
120	11.5	22
150	11.2	25
150	23.7	23

TABLE 16

Data for section 4.7.3.

Styrene-7,8-oxide kinetics in blood after intraperitoneal application; rat

200 mg/kg:

AUC in blood calculated by:	e-functions [$Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t} + C_3 \times e^{\lambda_3 t}$]	trapezoidal rule
C_1 [ng/ml]	-145,600	
C_2 [ng/ml]	110,000	
C_3 [ng/ml]	1,000	
λ_1 [h^{-1}]	-12	
λ_2 [h^{-1}]	- 4.0	
λ_3 [h^{-1}]	- 1.7	
AUC* [ng × h/ml]	16,487	19,680

*: Area under the curve

TABLE 17

Data for section 4.7.3.

Styrene-7,8-oxide kinetics in blood after intraperitoneal application; mouse

100 mg/kg:

AUC in blood calculated by:	e-functions [$Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t} + C_3 \times e^{\lambda_3 t}$]	trapezoidal rule
C_1 [ng/ml]	80,000	
C_2 [ng/ml]	40,667	
C_3 [ng/ml]	12.2	
λ_1 [h^{-1}]	-18	
λ_2 [h^{-1}]	- 6.5	
λ_3 [h^{-1}]	- 1.4	
AUC* [ng × h/ml]	2,708	1,416

*: Area under the curve

TABLE 17 (continued)

Data for section 4.7.3.

Styrene-7,8-oxide kinetics in blood after intraperitoneal application; mouse

200 mg/kg:

AUC in blood calculated by:	e-functions $[Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t} + C_3 \times e^{\lambda_3 t}]$	trapezoidal rule
C_1 [ng/ml]	-500,000	
C_2 [ng/ml]	-214,100	
C_3 [ng/ml]	170	
λ_1 [h^{-1}]	-18	
λ_2 [h^{-1}]	- 6.8	
λ_3 [h^{-1}]	- 1.7	
AUC* [ng × h/ml]	11,782	12,190

*: Area under the curve

TABLE 17 (continued)

Data for section 4.7.3.

Styrene-7,8-oxide kinetics in blood after intraperitoneal application; mouse

500 mg/kg:

AUC in blood calculated by:	e-functions $[Y = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}]$	trapezoidal rule
C_1 [ng/ml]	-2,200,642	
C_2 [ng/ml]	1,944,164	
λ_1 [h^{-1}]	-6.8	
λ_2 [h^{-1}]	-5.1	
AUC* [ng × h/ml]	67,443	58,000

*: Area under the curve

TABLE 18

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); rat
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 20 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
20 ppm:		
0	7.7	266
1	3.4	250
2	2.9	256
19.3 ppm:		
0	32	224
1	15	216
2	1.4	195

Mean exposure concentration: 260 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
251 ppm:		
0	235	232
1	138	236
2	89	198
3	72	232
270 ppm:		
0	182	208
1	158	223
2	102	210
3	92	218

TABLE 18 (continued)

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); rat
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 350 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
349 ppm:		
0	338	210
1	144	172
354 ppm:		
0	283	236
1	175	228
2	136	234
3	110	214

Mean exposure concentration: 510 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
510 ppm:		
0	384	196
1	246	194
2	253	194
3	164	210
514 ppm:		
0	156	210
1	138	217
2	108	212
3	95	198

TABLE 18 (continued)

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); rat
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 800 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
805 ppm:		
0	460	206
1	282	208
2	203	208
790 ppm:		
0	251	234
1	220	219
2	284	230
3	203	238
788 ppm:		
0	296	204
1	295	230
2	175	208

TABLE 18 (continued)

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); rat
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 800 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
805 ppm:		
0	460	206
1	282	208
2	203	208
790 ppm:		
0	251	234
1	220	219
2	284	230
3	203	238
788 ppm:		
0	296	204
1	295	230
2	175	208

TABLE 19

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); mouse
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 20 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
19.8 ppm:		
0	9	24
1	0.2	23
20.2 ppm:		
0	7.8	24
22 ppm:		
0	5.3	27
0.5	1.3	27
0.83	1.5	28
19.5 ppm:		
0	6.5	27
0.25	2.5	26
0.5	0.7	27
0.83	0.6	27

Mean exposure concentration: 260 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
245 ppm:		
0	44	24
1	7.2	23
2	5.4	25
3	8.4	24
256 ppm:		
0	282	22
1	4.6	24
2	5.1	22
3	11	22
264 ppm:		
0	272	28
1	29	25
2	1	27

TABLE 19 (continued)

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); mouse
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 360 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
367 ppm:		
0	1,476	26
1	234	27
2	10.1	26
3	5.7	24
350 ppm:		
0	3,800	23
1	200	24
2	69	23
3	22	22
358 ppm:		
0	2,100	28
1	233	28
2	11	30

Mean exposure concentration: 530 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
540 ppm:		
0	1,436	26
1	665	24
2	86	25
3	9.1	26
520 ppm:		
0	4,657	23
1	3,708	23
2	814	23
520 ppm:		
0	3,880	29
1	1,410	29
2	133	27
3	48	32

TABLE 19 (continued)

Data for section 4.7.4.

Styrene-7,8-oxide in blood after exposure to constant atmospheric styrene (steady-state conditions); mouse
Each data point comes from one animal; see note section 4.7.4.; page 34

Mean exposure concentration: 800 ppm

Time [h]	Conc. [ng SO/ml blood]	Body weight [g]
797 ppm:		
0	3,856	27
1	3,303	28
2	1,927	28
3	643	25
801 ppm:		
0	5,617	20
1	5,471	20
2	3,486	20
3	2,071	23
799 ppm:		
0	7,540	28
1	6,160	27
2	5,840	29
3	3,210	32

TABLE 20

Data for section 4.6.

Kinetics of styrene-7,8-oxide

Hydrolysis in aqueous systems

Hydrolysis of styrene-7,8-oxide kinetics
in aqueous systems (37 °C)

Time [min]	So concentration [%] in HCl pH 3	SO concentration [%] in HCl pH 4	SO concentration [%] in aqua bidest. pH 6.2	SO concentration [%] in buffer* pH 6	SO concentration [%] in buffer* pH 7
0	100.00	100.00	100.00	100.00	100.00
1	16.94	76.02		87.53	98.96
30		0.08			
120				33.88	77.44
180			79.41		
180			78.12		
210				13.76	62.82
300			57.53	6.18	51.53
360			53.18		
420			34.24	2.02	38.12
960			18.71		
1,200			26.00		
1,440			10.82		
1,440			12.47		

* Potassium phosphate buffer (200 mmol/l)

TABLE 21

Section 4.2.

Determination of partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide in vitro; rat

Tissue = FAT

	Exp.1[1]	Exp.2[1]	Exp.3[1]	Exp.4[1]	Exp.5[1]
Blood	$Y_1 = C \times e^{-k_{el}t}$ $AUC_B = C/k_{el}$				
C (peak area)	970	1042	614	925	446
k_{el} (h^{-1})	0.62	0.65	0.69	0.45	0.57
AUC_B (peak area × h)	1569	1596	896	2037	782
Tissue + Blood	$Y_1 = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}$ $AUC_{T+B} = C_1/\lvert\lambda_1\rvert + C_2/\lvert\lambda_2\rvert$				
C_1 (peak area)	383	556	325	403	238
$-\lambda_1$ (h^{-1})	2.76	2.23	2.21	1.42	1.22
C_2 (peak area)	597	488	337	529	210
$-\lambda_2$ (h^{-1})	0.55	0.40	0.59	0.41	0.43
AUC_{T+B} (peak area × h)	1225	1475	718	1580	683
AUC_{T+B}/AUC_B	0.78	0.92	0.80	0.78	0.87
	0.83 ± 0.06				
$P_{T/B}$ [2] = $P_{Fat/B}$	5.45	6.27	7.48	6.16	5.18
$P_{Fat/B}$; mean ± SD	6.1 ± 0.9				

[1] Data were obtained by GC/FID measurement
[2] Equations for calculating PT/B are given in Section 4.2.

TABLE 22

Section 4.2.

Determination of partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide in vitro; mouse

Tissue = FAT

		Exp. 1 [1]	Exp. 2 [1]	Exp. 3 [1]
Blood		$Y_1 = C \times e^{-k_{el}t}$ $AUC_B = C/k_{el}$		
C	(peak area)	540454	583301	577424
k_{el}	(h^{-1})	0.28	0.25	0.27
AUC_B	(peak area × h)	1930193	2333204	2062229
Tissue + Blood		$Y_1 = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}$ $AUC_{T+B} = C_1/\lvert\lambda_1\rvert + C_2\lvert\lambda_2\rvert$		
C_1	(peak area)	194288	174489	178598
$-\lambda_1$	(h^{-1})	3.70	2.98	2.48
C_2	(peak area)	335782	404875	391901
$-\lambda_2$	(h^{-1})	0.24	0.23	0.25
AUC_{T+B}	(peak area × h)	1475329	1788764	1621031
AUC_{T+B}/AUC_B		0.76	0.77	0.79
			0.77 ± 0.03	

$P_{T/B}$ [2] = $P_{Fat/B}$	5.42	4.19	4.27
$P_{Fat/B}$; mean ± SD		4.6 ± 0.69	

[1] Data were obtained by GC/MSD measurement
[2] Equations for calculating PT/B are given in Section 4.2.

Experiment 1 was carried out with male B6C3F1 mice, Experiments 2 and 3 with male NMRI mice.

TABLE 23

Section 4.2.

Determination of partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide in vitro; rat

Tissue - MUSCLE

		Exp. 1 [1]	Exp. 2 [1]	Exp. 3 [1]	Exp. 4 [1]
Blood		$Y_1 = X \times e^{-k_{el}t}$ $AUC_B = C/k_{el}$			
C	(peak area)	634372	564177	328	422
k_{el}	(h^{-1})	0.56	0.54	0.48	0.59
AUC_B	(peak area × h)	1130788	1039000	683	714
Tissue + Blood		$Y_1 = C_1 \times e^{\lambda_2 t} + C_2 \times e^{\lambda_2 t}$ $AUC_{T+B} = C_1/\lvert\lambda_1\rvert + C_2/\lvert\lambda_2\rvert$			
C_1	(peak area)	272199	286005	184	251
$-\lambda_1$	(h^{-1})	3.59	4.01	1.21	3.77
C_2	(peak area)	346064	273166	146	179
$-\lambda_2$	(h^{-1})	0.37	0.33	0.30	0.39
AUC_{T+B}	(peak area × h)	106144	906782	632	533
AUC_{T+B}/AUC_B		0.89	0.87	0.93	0.75
		0.86 ± 0.08			
$P_{T/B}$ [3] = $P_{Mus/B}$		1.18	1.60	1.08	2.20
$P_{Mus/B}$; mean ± SD		1.5 ± 0.51			

[1] Data were obtained by GC/MSD measurement

[2] Data were obtained by GC/FID measurement

[3] Equations for calculating PT/B are given in Section 4.2.

TABLE 24

Section 4.2.

Determination of partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide in vitro; mouse

Tissue - MUSCLE

		Exp. 1 [1]	Exp. 2 [1]	Exp. 3 [1]
Blood		$Y_1 = C \times e^{-k_{el}t}$ $AUC_B = C/k_{el}$		
C	(peak area)	570438	521394	587165
k_{el}	(h^{-1})	0.35	0.22	0.24
AUC_B	(peak area × h)	1629823	2369973	2446521
Tissue + Blood		$Y_1 = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}$ $AUC_{T+B} = C_1/\lvert\lambda_1\rvert + C_2\lvert\lambda_2\rvert$		
C_1	(peak area)	187839	202487	227275
$-\lambda_1$	(h^{-1})	1.42	2.29	3.62
C_2	(peak area)	324354	311028	385003
$-\lambda_2$	(h^{-1})	0.30	0.19	0.22
AUC_{T+B}	(peak area × h)	1218069	1733957	1781616
AUC_{T+B}/AUC_B		0.75	0.73	0.73
			0.74 ± 0.01	
$P_{T/B}$ [2] = $P_{Mus/B}$		0.89	1.22	1.15
$P_{Mus/B}$; mean ± SD			1.1 ± 0.18	

[1] Data were obtained by GC/MSD measurement
[2] Equations for calculating PT/B are given in Section 4.2.

Experiment 1 was carried out with male B6C3F1 mice, Experiments 2 and 3 with male NMRI mice.

TABLE 25

Section 4.2.

Determination of partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide in vitro; rat

Tissue - LIVER

		Exp. 1 [1]	Exp. 2 [1]	Exp. 3 [1]
Blood		$Y_1 = C \times e^{-k_{el}t}$ $AUC_B = C/k_{el}$		
C	(peak area)	667756	597728	581074
k_{el}	(h^{-1})	0.56	0.45	0.63
AUC_B	(peak area × h)	1200999	1325337	926753
Tissue + Blood		$Y_1 = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}$ $AUC_{T+B} = C_1/\lvert\lambda_1\rvert + C_2\lvert\lambda_2\rvert$		
C_1	(peak area)	481035	318639	313016
$-\lambda_1$	(h^{-1})	1.72	5.51	4.43
C_2	(peak area)	148477	252123	264087
$-\lambda_2$	(h^{-1})	0.26	0.31	0.41
AUC_{T+B}	(peak area × h)	854677	876452	714638
AUC_{T+B}/AUC_B		0.71	0.66	0.77
			0.71 ± 0.05	

$P_{T/B}$ [2] = $P_{Liv/B}$	3.49	2.28	1.91
$P_{Liv/B}$; mean ± SD		2.6 ± 0.83	

[1] Data were obtained by GC/FID measurement
[2] Equations for calculating PT/B are given in Section 4.2.

TABLE 26

Section 4.2.

Determination of partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide in vitro; rat

Tissue - ESOPHAGUS

		Exp. 1 [1]	Exp. 2 [1]	Exp. 3 [1]
Blood		$Y_1 = C \times e^{-k_{el}t}$ $AUC_B = C/k_{el}$		
C	(peak area)	1006	1233	1083
k_{el}	(h^{-1})	0.55	0.48	0.62
AUC_B	(peak area × h)	1836	2569	1755
Tissue + Blood		$Y_1 = C_1 \times e^{\lambda_1 t} + C_2 \times e^{\lambda_2 t}$ $AUC_{T+B} = C_1/\lvert\lambda_1\rvert + C_2\lvert\lambda_2\rvert$		
C_1	(peak area)	238	294	418
$-\lambda_1$	(h^{-1})	4.97	3.52	2.59
C_2	(peak area)	797	886	674
$-\lambda_2$	(h^{-1})	0.46	0.37	0.38
AUC_{T+B}	(peak area × h)	1788	2465	1821
AUC_{T+B}/AUC_B		0.97	0.96	1.04
			0.99 ± 0.03	

$P_{T/B}$ [2] = $P_{Oes/B}$	2.01	1.97	2.59
$P_{Oes/B}$; mean ± SD		2.2 ± 0.35	

[1] Data were obtained by GC/FID measurement
[2] Equations for calculating PT/B are given in Section 4.2.

TABLE 27

Data for section 4.3.

Inhalation kinetics with styrene; rat; without pretreatment

Application:	into chamber (liquid)
Initial conc. in chamber:	about 700 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	404 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	274.000
2	0.100	362.000
3	0.317	224.000
4	0.633	235.000
5	1.183	156.000
6	1.567	128.000
7	2.017	72.000
8	2.533	52.000
9	2.983	29.000
10	3.817	13.000
11	4.617	4.000
12	5.583	2.000

TABLE 27 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; without pretreatment

Application:	into chamber (liquid)
Initial conc. in chamber:	about 700 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	410 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	217.000
2	0.100	261.000
3	0.167	321.000
4	0.233	362.000
5	0.317	338.000
6	0.517	357.000
7	1.083	238.000
8	1.817	143.000
9	2.717	70.000
10	3.617	15.000
11	4.700	6.000
12	5.633	2.000

TABLE 27 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 1,100 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	393 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	1,036.000
2	0.100	760.000
3	0.167	1,083.000
4	0.250	911.000
5	0.317	811.000
6	0.817	839.000
7	1.450	246.000
8	1.767	208.000
9	2.633	111.000
10	3.617	49.000
11	4.567	22.000
12	5.583	9.000
13	6.567	3.000
14	7.583	2.000
15	8.650	1.000

TABLE 27 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 1,600 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	388 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	1,572.000
2	0.133	1,220.000
3	0.217	1,086.000
4	0.283	1,095.000
5	0.350	1,191.000
6	0.550	510.000
7	1.400	464.000
8	1.933	330.000
9	2.833	199.000
10	3.733	106.000
11	4.583	52.000
12	5.600	21.000
13	6.617	10.000
14	7.817	5.000
15	8.867	2.000
16	9.017	2.000

TABLE 27 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 3,000 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	397 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.003	2,990.00
2	0.08	3,060.00
3	0.17	2,383.00
4	0.23	1,828.00
5	0.32	2.093.00
6	0.47	1.717.00
7	1.42	821.00
8	2.22	343.00
9	2.97	214.00
10	3.90	149.00
11	4.72	104.00
12	5.80	61.00
13	6.85	45.00
14	7.82	30.00
15	8.85	16.00
16	9.83	7.00
17	11.08	4.00
18	12.00	2.00

TABLE 27 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 3,100 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	444 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	3,034.000
2	0.133	2,874.000
3	0.200	2,837.000
4	0.267	2,724.000
5	0.333	2,367.000
6	1.200	1,301.000
7	2.267	489.000
8	3.067	236.000
9	4.067	138.000
10	4.933	92.000
11	5.867	82.000
12	7.100	54.000
13	7.867	41.000
14	8.967	17.000
15	9.033	19.000
16	10.017	7.000
17	11.083	7.000
18	12.033	1.200
19	13.050	0.800
20	14.017	0.600
21	15.033	1.900
22	15.100	0.500
23	16.000	0.300
24	16.967	0.200

TABLE 28

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 780 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	124 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	784.000
2	0.117	766.000
3	0.183	801.000
4	0.267	726.000
5	0.700	491.000
6	1.083	445.000
7	1.583	278.000
8	2.667	202.000
9	3.083	147.000
10	3.983	82.000
11	5.050	45.000
12	5.783	31.000
13	7.050	15.000
14	8.050	11.000
15	9.100	8.200
16	9.250	6.900

TABLE 28 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 1,100 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	123 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	1,078.000
2	0.117	1,120,000
3	0.183	1,094.000
4	0.233	1,110.000
5	0.317	1,103.000
6	0.400	1,014.000
7	0.900	868.000
8	1.483	525.000
9	1.933	386.000
10	2.633	278.000
11	3.867	146.000
12	4.150	139.000
13	5.017	68.000
14	6.083	35.000
15	6.867	20.000
16	8.117	12.000
17	9.000	5.800
18	10.050	4.900
19	10.183	2.600

TABLE 28 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 2,300 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	128 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	2,252.000
2	0.117	2,158,000
3	0.183	2,129.000
4	0.267	2,168.000
5	0.333	1,881.000
6	1.183	1,297.000
7	2.083	648.000
8	3.017	438.000
9	4.050	258.000
10	5.067	186.000
11	6.533	98.000
12	7.183	66.000
13	8.083	44.000
14	9.033	22.000
15	9.967	13.000

TABLE 28 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 2,900 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	128 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	2,940.000
2	0.150	2,775,000
3	0.267	2,432.000
4	0.350	2,265.000
5	1.033	1,339.000
6	2.183	649.000
7	3.233	470.000
8	4.017	318.000
9	5.033	209.000
10	6.067	121.000
11	7.483	83.000
12	8.200	60.000
13	9.100	31.000
14	10.050	18.000

TABLE 28 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 4,600 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	117 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	3,519.000
2	0.150	4,562,000
3	0.250	4,232.000
4	0.400	3,959.000
5	0.567	3,367.000
6	1.350	2,316.000
7	1.950	1,966.000
8	3.050	1,184.000
9	3.300	1,173.000
10	4.033	1,015.000
11	5.067	728.000
12	6.067	583.000
13	7.000	515.000
14	8.100	417.000
15	9.167	341.000
16	9.967	365.000

TABLE 28 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 4,600 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	117 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	4,626.000
2	0.167	4,031.000
3	0.283	3,448.000
4	0.367	3,502.000
5	1.167	2,381.000
6	2.100	1,820.000
7	3.000	1,046.000
8	4.033	674.000
9	5.150	606.000
10	5.350	623.000
11	6.083	524.000
12	7.217	375.000
13	8.150	392.000
14	8.250	341.000
15	9.033	296.000
16	10.050	235.000
17	10.633	214.000
18	11.083	200.000
19	12.050	162.000

TABLE 28 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; without pretreatment

Application:	into chamber (gas)
Initial conc. in chamber:	about 3,900 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	129 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	3,170.000
2	0.150	3,674.000
3	0.233	3,915.000
4	0.417	3,579.000
5	0.533	3,506.000
6	0.650	3,354.000
7	1.150	2,291.000
8	1.750	1,529.000
9	2.267	1,142.000
10	2.917	1,027.000
11	4.033	708.000
12	5.117	470.000
13	6.150	400.000
14	7.000	317.000
15	8.150	254.000
16	9.100	258.000
17	10.200	200.000
18	11.200	163.000

TABLE 29

Data for section 4.3.

Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	Dithiocarb (200 mg/kg body weight, 15 min. prior to exposure)
Application:	into chamber (gas)
Initial conc. in chamber:	about 1,300 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	379 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	1,235.000
2	0.117	1,131.000
3	0.183	1,096.000
4	0.250	1,012.000
5	0.317	1,010.000
6	0.383	916.000
7	0.950	548.000
8	2.067	170.000
9	2.817	136.000
10	4.000	82.000
11	5.150	65.000
12	6.000	63.000
13	7.050	54.000
14	8.050	52.000
15	9.050	50.000
16	10.033	50.000
17	11.050	47.000
18	12.033	33.000
19	13.067	35.000
20	14.000	39.000
21	15.000	30.000
22	16.017	24.000
23	17.100	22.000
24	17.983	17.000
25	18.033	18.000

TABLE 30
Data for section 4.3.
Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	Dithiocarb (400 mg/kg body weight, 15 min. prior to exposure)
Application:	into chamber (gas)
Initial conc. in chamber:	about 1,400 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	141 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.05	1.658
2	0.1	1,298
3	0.15	1,454
4	0.267	1,146
5	0.4	1,088
6	1.017	805
7	2.117	480
8	3.233	317
9	3.967	244
10	4.983	164
11	6.183	138
12	6.633	132
13	7.75	131
14	8.183	125
15	9.117	136
16	9.85	103
17	11.083	83
18	12.033	75

TABLE 31
Data for section 4.3.
Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	about 150 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,300 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	538 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	4,343.000
2	0.083	3,672,000
3	0.167	3,134.000
4	0.267	3,387.000
5	0.750	1,742.000
6	1.333	846.000
7	1.867	432.000
8	2.950	197.000
9	3.850	142.000
10	4.817	104.000
11	5.700	88.000
12	6.717	72.000
13	7.683	55.000
14	8.850	37.000
15	9.867	27.000
16	10.817	20.000
17	11.550	15.000
18	11.967	9.000

TABLE 31 (continued)
Data for section 4.3.
Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	about 150 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 5,200 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	530 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	5,214.000
2	0.083	3,917.000
3	0.150	3,663.000
4	0.383	3,507.000
5	0.833	1,427.000
6	1.467	603.000
7	2.533	268.000
8	3.433	143.000
9	4.317	103.000
10	5.300	79.000
11	6.267	65.000
12	7.267	50.000
13	8.433	37.000
14	9.467	28.000
15	10.250	21.000
16	11.067	14.000
17	11.800	9.000
18	12.100	7.000

TABLE 31 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	about 150 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,800 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	530 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	4,819.000
2	0.233	2,522.000
3	0.300	2,054.000
4	0.400	2,293.000
5	1.150	879.000
6	2.233	261.000
7	3.117	154.000
8	3.617	115.000
9	4,983	69.000
10	5.583	64.000
11	5.933	64.000
12	6.583	43.000
13	7.400	35.000
14	8.117	31.000
15	8.667	25.000
16	9.483	19.000
17	10.767	10.000
18	11.433	9.000
19	11.833	4.000
20	11.883	4.000

TABLE 32
Data for section 4.3.
Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	about 500 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,400 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	461 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	4,431.000
2	0.083	3,485.000
3	0.217	3,034.000
4	0.633	1,742.000
5	1.350	939.000
6	2.400	300.000
7	3.317	193.000
8	4.083	118.000
9	5.400	84.000
10	6.017	68.000
11	7.017	46.000
12	8.017	35.000
13	8.967	26.000
14	10.050	15.000
15	10.733	9.000
16	11.483	5.000
17	12.000	4.000
18	12.033	4.000

TABLE 32 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	about 500 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,300 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	457 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	4,285.000
2	0.117	3,219.000
3	0.217	2,780.000
4	0.667	1,940.000
5	1.450	702.000
6	2.683	232.000
7	3.633	134.000
8	4.333	96.000
9	5.717	53.000
10	6.300	39.000
11	6.333	49.000
12	7.317	36.000
13	8.333	27.000
14	9.283	14.000
15	10.367	7.000
16	11.050	4.000
17	11.800	3.000
18	12.050	1.000
19	12.083	2.000

TABLE 32 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; rat; with pretreatment

Pretreatment:	about 500 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 5,100 ppm
Initial conc. in animal:	0 ppm
Species:	rat
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	461 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.017	5,085.000
2	0.100	3,429.000
3	0.167	2,893.000
4	0.267	2,656.000
5	0.767	1,599.000
6	1.233	879.000
7	1.750	507.000
8	3.133	150.000
9	4.083	93.000
10	4.900	74.000
11	6.150	42.000
12	6.750	39.000
13	7.783	25.000
14	8.767	13.000
15	9.700	8.000
16	10.817	3.000
17	11.450	2.000
18	11.500	2.000
19	12.017	1.700
20	12.050	0.800
21	12.083	1.700

TABLE 33
Data for section 4.3.
Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	about 150 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,800 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	116 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	4,836.000
2	0.100	3,862.000
3	0.250	3,207.000
4	0.417	2,781.000
5	0.833	2,012.000
6	1.500	1,242.000
7	2.133	921.000
8	3.183	631.000
9	3.900	449.000
10	4.850	325.000
11	5.933	277.000
12	6.900	208.000
13	7.633	189.000
14	8.717	145.000
15	9.817	117.000
16	10.883	93.000
17	11.683	96.000
18	11.817	79.000

TABLE 33 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	about 150 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,100 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	122 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	4,081.000
2	0.150	3,175.000
3	0.233	3,384.000
4	0.900	1,531.000
5	1.667	1,073.000
6	2.733	642.000
7	3.333	494.000
8	4.450	378.000
9	5.467	264.000
10	6.417	203.000
11	7.150	156.000
12	8.233	124.000
13	9.333	87.000
14	10.400	66.000
15	11.183	48.000
16	12.017	35.000

TABLE 33 (continued)
Data for section 4.3.
Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	about 150 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 4,800 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	112 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	4,810.000
2	0.150	3,535.000
3	0.233	3,647.000
4	0.317	2,882.000
5	0.433	2,873.000
6	0.750	2,261.000
7	1.117	1,992.000
8	2.450	946.000
9	2.917	822.000
10	3.867	560.000
11	4.867	411.000
12	6.117	337.000
13	6.917	303.000
14	7.950	229.000
15	9.033	190.000
16	10.083	159.000
17	10.883	130.000
18	12.000	112.000

TABLE 34
Data for section 4.3.
Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	about 500 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 3,600 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	124 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	3,641.000
2	0.117	2,826.000
3	0.183	2,573.000
4	0.250	2,273.000
5	0.767	1,406.000
6	1.283	862.000
7	1.750	643.000
8	2.617	428.000
9	3.350	336.000
10	4.433	238.000
11	5.250	173.000
12	6.183	129.000
13	7.150	81.000
14	8.183	52.000
15	9.183	35.000
16	10.183	25.000
17	11.017	17.000
18	12.000	11.000

TABLE 34 (continued)
Data for section 4.3.
Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	about 500 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 3,800 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	120 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	3,754.000
2	0.117	2,623.000
3	0.200	2,831.000
4	0.283	2,432.000
5	0.917	1,507.000
6	1.267	1,087.000
7	2.150	621.000
8	2.983	476.000
9	4.067	312.000
10	4.867	219.000
11	5.817	176.000
12	6.767	133.000
13	7.850	93.000
14	8.833	80.000
15	9.817	56.000
16	10.633	39.000
17	11.483	26.000
18	12.050	20.000

TABLE 34 (continued)

Data for section 4.3.

Inhalation kinetics with styrene; mouse; with pretreatment

Pretreatment:	about 500 ppm styrene, 5 days, 6 h/d
Application:	into chamber (gas)
Initial conc. in chamber:	about 3,200 ppm
Initial conc. in animal:	0 ppm
Species:	mouse
Sex:	male
Number of animal(s):	5
Vol. of animal(s):	130 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	3,212.000
2	0.100	2,663.000
3	0.183	2,572.000
4	0.267	2,340.000
5	0.383	2,142.000
6	0.567	1,664.000
7	0.800	1,456.000
8	1.700	760.000
9	2.317	583.000
10	3.700	372.000
11	4.517	269.000
12	5.450	223.000
13	6.400	154.000
14	7.483	117.000
15	8.450	86.000
16	9.450	72.000
17	10.283	50.000
18	11.117	38.000
19	11.600	29.000
20	12.000	26.000

TABLE 35

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	5,435 ppm; 5 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	202 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	0.800
2	0.117	1.100
3	0.167	1.600
4	0.217	2.000
5	0.300	2.400
6	0.350	2.700
7	0.400	2.800
8	0.450	3.300
9	0.500	3.500
10	0.633	3.700
11	0.717	4.100
12	0.783	4.400
13	0.833	4.400
14	0.900	4.400
15	0.950	4.300
16	1.067	4.200
17	1.500	3.000
18	1.950	1.800
19	3.117	1.000

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	5,382 ppm; 5 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	204 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	0.900
2	0.067	1.000
3	0.183	1.300
4	0.250	1.700
5	0.317	2.100
6	0.367	2.300
7	0.433	2.600
8	0.600	3.400
9	0.750	3.400
10	0.917	4.300
11	1.133	4.200
12	1.183	4.200
13	1.617	3.100
14	1.750	3.000
15	1.950	3.000
16	2.150	2.600
17	2.367	2.000
18	2.817	1.600

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	10,260 ppm (equivalent nl gas/ml tissue); 10 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	214 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	7.000
2	0.133	7.000
3	0.167	8.000
4	0.217	8.000
5	0.267	8.000
6	0.317	9.000
7	0.367	10.000
8	0.433	11.000
9	0.483	10.000
10	0.533	12.000
11	0.583	12.000
12	0.650	13.000
13	0.700	13.000
14	0.750	14.000
15	0.817	14.000
16	0.867	14.000
17	0.933	15.000
18	0.983	15.000
19	1.033	14.000
20	1.083	13.000
21	1.133	14.000
22	1.200	13.000
23	1.267	13.000
24	1.717	9.000
25	2.050	9.000
26	2.650	6.000
27	3.083	5.000
28	4.100	4.000
29	5.100	2.000
30	6.033	2.000

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	9,980 ppm; 10 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	220 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	21.000
2	0.100	23.000
3	0.167	22.000
4	0.233	22.000
5	0.283	23.000
6	0.533	21.000
7	0.600	21.000
8	0.650	22.000
9	0.850	16.000
10	0.933	14.000
11	1.000	15.000
12	1.100	14.000
13	1.183	14.000
14	1.933	7.000
15	1.983	7.000
16	2.950	4.000
17	3.700	3.000
18	4.150	3.000
19	5.067	2.000
20	6.067	0.900

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	11,089 ppm; 10 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	198 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	1.000
2	0.117	2.000
3	0.167	2.000
4	0.217	3.000
5	0.250	3.000
6	0.300	4.000
7	0.417	4.000
8	0.483	6.000
9	0.667	7.000
10	0.717	6.000
11	0.800	9.000
12	0.867	8.000
13	0.967	8.000
14	1.017	10.000
15	1.083	11.000
16	1.133	10.000
17	1.200	9.000
18	1.283	10.000
19	1.417	10.000
20	1.467	10.000
21	1.617	10.000
22	1.683	11.000
23	2.667	9.000
24	3.050	7.000
25	4.033	5.000
26	4.983	2.000
27	6.017	1.000
28	7.167	0.900

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Exposure No. 27

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	49,677 ppm
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	221 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.100
2	0.117	0.400
3	0.167	2.400
4	0.233	4.800
5	0.283	7.100
6	0.517	10.500
7	0.650	13.900
8	0.767	15.400
9	0.933	18.300
10	1.450	24.300
11	1.667	22.000
12	1.817	24.400
13	2.217	30.000
14	2.383	32.100
15	2.933	33.100
16	3.467	35.400
17	4.200	34.100
18	5.467	35.400
19	6.083	34.700
20	6.983	27.700
21	8.050	20.700
22	9.033	14.400
23	10.033	7.500
24	11.267	2.600
25	11.333	2.400
26	12.000	1.900
27	13.017	1.000

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Exposure No. 29

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	49,453 ppm
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	222 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.083	1.500
2	0.150	3.400
3	0.200	6.100
4	0.250	9.800
5	0.300	10.500
6	0.500	22.000
7	0.633	34.500
8	0.783	43.700
9	0.917	53.000
10	1.217	61.400
11	1.450	75.800
12	1.567	72.100
13	1.950	62.600
14	2.033	69.700
15	2.400	86.300
16	3.150	85.800
17	4.450	70.300
18	5.050	67.900
19	6.000	52.400
20	7.033	37.600
21	8.000	32.400
22	8.067	33.600
23	8.983	19.200
24	10.033	12.900
25	11.000	6.100
26	12.017	3.500
27	12.867	2.400

TABLE 35 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to rats

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	48,794 ppm; 50 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	225 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	0.400
2	0.100	1.700
3	0.150	2.400
4	0.200	4.000
5	0.283	5.800
6	0.367	7.200
7	0.533	10.000
8	0.617	11.500
9	0.917	14.800
10	1.083	14.400
11	1.333	20.700
12	2.233	22.900
13	3.250	35.200
14	3.333	31.600
15	3.900	32.900
16	5.017	36.100
17	5.933	44.600
18	6.017	35.800
19	6.950	38.000
20	7.117	40.800
21	8.100	34.900
22	9.100	34.900
23	9.983	29.900
24	11.117	18.800
25	11.967	13.500
26	12.983	7.500
27	14.000	3.800
28	15.017	2.000
29	16.000	1.200
30	16.967	0.700

TABLE 36

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to mice

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	45,800 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	48 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	0.60
2	0.10	0.60
3	0.13	0.70
4	0.53	2.90
5	0.77	5.00
6	0.88	6.00
7	1.02	7.80
8	1.18	8.50
9	1.47	11.30
10	1.80	12.60
11	2.07	12.70
12	2.90	10.50
13	3.62	8.30
14	4.25	6.70
15	4.85	5.90
16	5.65	4.40
17	6.05	3.80
18	7.10	2.80
19	8.05	2.00
20	8.43	0.90

TABLE 36 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to mice

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	43,400 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	50 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	19.000
2	0.117	17.000
3	0.167	17.000
4	0.283	18.000
5	0.350	18.000
6	0.467	15.000
7	1.450	16.000
8	2.500	12.000
9	3.350	9.000
10	4.050	6.900
11	4.717	5.300
12	5.400	3.900
13	5.983	3.700
14	6.083	3.200
15	6.400	3.200
16	6.583	3.100
17	7.000	2.600
18	8.333	1.800
19	8.933	1.200

TABLE 36 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to mice

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	41,400 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	53 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.133	0.300
2	0.217	0.400
3	0.333	1.500
4	0.433	2.200
5	0.567	3.300
6	0.700	4.400
7	0.833	5.800
8	1.150	7.800
9	1.450	8.800
10	1.750	10.000
11	1.933	9.600
12	2.600	8.400
13	3.317	6.400
14	3.950	5.400
15	4.533	4.400
16	5.267	3.100
17	5.700	2.900
18	6.750	2.200
19	7.783	1.600
20	8.300	1.300

TABLE 36 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to mice

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	75,714 ppm; 10 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	58 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	2.300
2	0.117	5.100
3	0.183	7.200
4	0.250	8.600
5	0.333	10.400
6	0.433	16.100
7	0.500	14.200
8	0.567	14.500
9	0.750	16.100
10	0.850	15.800
11	0.967	16.300
12	1.400	16.800
13	2.117	14.300
14	2.500	15.000
15	3.050	14.000
16	4.133	12.400
17	5.150	9.600
18	6.583	8.000
19	7.217	7.600
20	8.250	6.700
21	9.150	6.200
22	10.117	5.100
23	11.383	4.700
24	12.067	3.800

TABLE 36 (continued)

Data for section 4.4.

Kinetics of styrene after intraperitoneal application of styrene to mice

Application:	intraperitoneal (liquid)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	79,846 ppm; 10 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	55 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.083	6.500
2	0.150	10.600
3	0.183	13.400
4	0.417	23.000
5	0.583	25.000
6	0.750	30.000
7	0.967	35.000
8	1.500	35.600
9	2.567	33.600
10	3.567	33.300
11	4.100	33.300
12	5.050	27.800
13	6.000	23.600
14	7.067	20.000
15	7.900	19.900
16	9.133	15.400
17	9.967	11.600
18	10.983	8.800
19	11.483	9.000
20	11.533	8.300
21	11.633	7.000

TABLE 37

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	26,750 ppm; 24 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	197 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	3.400
2	0.100	3.800
3	0.183	4.100
4	0.217	4.400
5	0.500	6.100
6	1.033	7.900
7	1.333	9.000
8	1.767	8.800
9	2.950	7.300
10	4.083	6.400
11	5.183	5.700
12	6.400	4.500
13	7.050	2.800
14	8.017	2.300

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	29,114 ppm; 24 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	181 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.050	0.120
2	0.100	0.170
3	0.283	1.900
4	0.550	3.900
5	0.717	7.700
6	1.033	8.800
7	1.283	16.900
8	1.833	17.100
9	2.783	19.000
10	3.900	15.000
11	5.400	3.400
12	5.900	5.100
13	7.017	2.600
14	7.083	2.200
15	8.233	1.200

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	25,581 ppm; 24 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	206 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	2.100
2	0.117	2.500
3	0.217	3.900
4	0.283	6.100
5	0.500	10.400
6	1.000	15.800
7	1.583	18.900
8	2.300	15.800
9	3.333	12.000
10	4.600	5.700
11	5.733	2.800
12	6.417	1.700
13	7.367	1.200

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	20,995 ppm; 24 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	251 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.230
2	0.167	0.450
3	0.233	0.800
4	0.283	0.520
5	0.317	1.510
6	0.383	1.940
7	0.600	3.200
8	0.867	4.800
9	1.067	4.800
10	1.633	5.600
11	2.017	5.600
12	3.367	3.900
13	4.200	2.900
14	5.767	1.330

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	20,037 ppm; 24 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	263 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.110
2	0.117	0.120
3	0.167	0.380
4	0.200	0.620
5	0.350	1.900
6	0.550	3.600
7	0.900	7.300
8	1.100	6.000
9	1.333	7.400
10	2.750	7.000
11	3.750	4.000
12	5.200	1.200
13	5.600	0.900

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	40,850 ppm; 40 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	215 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.660
2	0.100	0.730
3	0.150	1.600
4	0.217	4.800
5	0.317	7.900
6	0.400	10.200
7	0.450	12.100
8	0.883	19.100
9	1.117	24.200
10	1.350	28.600
11	1.867	34.900
12	2.467	31.100
13	3.333	27.900
14	4.233	26.500
15	5.667	15.600
16	7.350	12.500
17	8.383	7.400
18	9.650	3.500
19	10.867	1.700
20	11.783	1.200
21	12.817	0.690

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	43,265 ppm; 40 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	203 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25°C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.07	0.03
2	0.15	0.61
3	0.20	1.50
4	0.23	1.90
5	0.40	5.80
6	0.48	7.10
7	0.63	12.00
8	0.70	13.60
9	1.12	22.10
10	1.63	23.80
11	1.93	22.30
12	2.98	19.80
13	3.60	21.20
14	5.38	15.60
15	6.68	10.50
16	7.73	6.40
17	8.98	3.90
18	10.00	2.50
19	11.12	2.10
20	12.12	2.50
21	12.17	0.97

TABLE 37 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to rats

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	43,265 ppm; 40 µl
Species:	rat
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	203 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.970
2	0.117	1.400
3	0.200	3.600
4	0.400	9.800
5	0.700	21.800
6	1.000	28.200
7	1.883	32.300
8	2.250	31.400
9	2.700	28.100
10	4.217	29.800
11	6.000	16.200
12	7.050	9.900
13	8.300	4.900
14	9.467	2.800
15	10.433	1.800
16	11.483	0.900
17	12.117	1.300
18	12.167	0.830

TABLE 38

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	38,100 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	57.6 ml
Vol. of chamber:	6,380 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.220
2	0.100	0.950
3	0.167	2.100
4	0.517	4.700
5	0.600	4.700
6	1.317	3.800
7	1.550	2.700
8	2.100	2.000
9	3.200	1.300
10	4.067	0.900
11	5.417	0.600
12	7.050	0.700
13	7.400	0.400
14	9.267	0.170

TABLE 38 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	37,900 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	57.8 ml
Vol. of chamber:	6,380 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.090
2	0.100	0.370
3	0.150	1.340
4	0.200	2.000
5	0.250	2.900
6	0.317	3.800
7	0.383	4.300
8	0.550	4.300
9	0.833	4.300
10	1.317	3.200
11	2.017	1.900
12	2.567	1.400
13	3.067	1.000
14	4.217	0.500
15	5.017	0.360
16	5.350	0.330
17	6.383	0.260
18	8.050	0.260
19	9.333	0.100

TABLE 38 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	38,500 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	57 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.083	2.300
2	0.167	3.400
3	0.217	4.100
4	0.267	4.700
5	0.350	4.900
6	0.450	6.000
7	0.533	6.700
8	0.600	6.900
9	0.700	6.800
10	0.983	6.100
11	1.483	5.600
12	2.100	4.000
13	3.067	4.000
14	3.367	3.900
15	3.833	3.300
16	5.133	2.400
17	6.167	2.000
18	7.067	1.400
19	8.033	1.100
20	9.150	0.850
21	10.000	0.700
22	11.083	0.550
23	12.033	0.430

TABLE 38 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	37,840 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	58 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.083	0.800
2	0.133	1.100
3	0.167	1.400
4	0.267	1.900
5	0.317	2.200
6	0.500	3.300
7	0.617	3.800
8	0.783	3.800
9	1.000	3.000
10	1.633	2.500
11	2.033	2.000
12	3.083	1.300
13	4.400	0.700
14	5.200	0.500
15	6.150	0.400
16	7.083	0.300
17	8.033	0.160

TABLE 38 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	39,900 ppm; 5 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	2
Vol. of animal(s):	55 ml
Vol. of chamber:	6,400 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.067	0.200
2	0.117	0.600
3	0.267	3.100
4	0.317	4.000
5	0.383	4.900
6	0.483	6.400
7	0.583	6.500
8	0.617	6.600
9	0.800	5.900
10	1.217	6.600
11	1.700	4.900
12	2.150	4.100
13	2.617	3.300
14	2.933	3.200
15	4.000	2.200
16	5.133	1.400
17	6.117	1.100
18	7.067	0.800
19	8.017	0.600
20	8.950	0.500
21	9.900	0.360
22	10.900	0.240

TABLE 38 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	87,800 ppm; 10 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	25 ml
Vol. of chamber:	830 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	1.500
2	0.067	2.000
3	0.217	2.400
4	0.367	8.500
5	0.600	10.000
6	1.000	7.800
7	1.183	6.700
8	1.917	1.900
9	2.733	0.460

TABLE 38 (continued)

Data for section 4.5.

Kinetics of styrene after oral application of styrene to mice

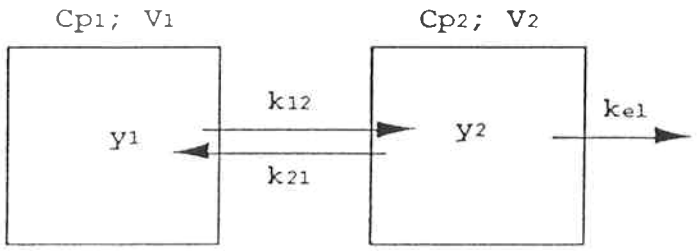
Application:	oral (liquid dissolved in olive oil)
Initial conc. in chamber:	0 ppm
Initial conc. in animal:	81,300 ppm; 10 µl per animal
Species:	mouse
Sex:	male
Number of animal(s):	1
Vol. of animal(s):	27 ml
Vol. of chamber:	850 ml
Temperature of chamber:	25 °C
Vol. of soda lime:	75 ml

Nr.	Time (h)	Concentration (ppm)
1	0.033	4.500
2	0.100	8.500
3	0.183	24.000
4	0.250	41.000
5	0.450	91.000
6	0.533	115.000
7	1.083	127.000
8	1.617	129.000
9	2.283	111.000
10	3.117	82.000
11	4.017	56.000
12	5.200	39.000
13	6.267	23.000
14	7.183	13.000
15	8.333	12.000
16	8.567	4.200
17	9.350	2.700

8. Figures

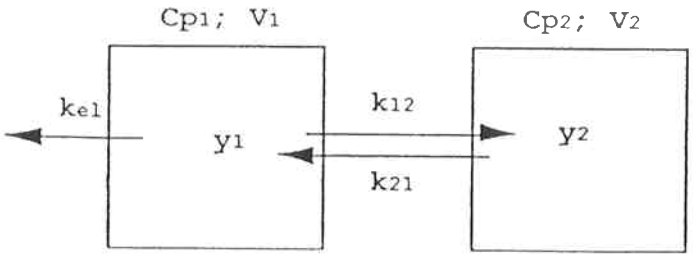
FIGURE 1

A. Pharmacokinetic two-compartment model for the determination of the partition coefficients "liquid/air" (PL/A) of styrene-7,8-oxide; Section 4.2.



Cp1; V1: compartment 1 = gas phase with volume 1
Cp2; V2: compartment 2 = liquid phase with volume 2
y1; y2: concentrations in Cp1 and Cp2
k12; k21: microconstants of the transport between the phases
kel: rate constant of the elimination

A. Pharmacokinetic two-compartment model for the determination of the partition coefficients "tissue/blood" (PT/B) of styrene-7,8-oxide; Section 4.2.

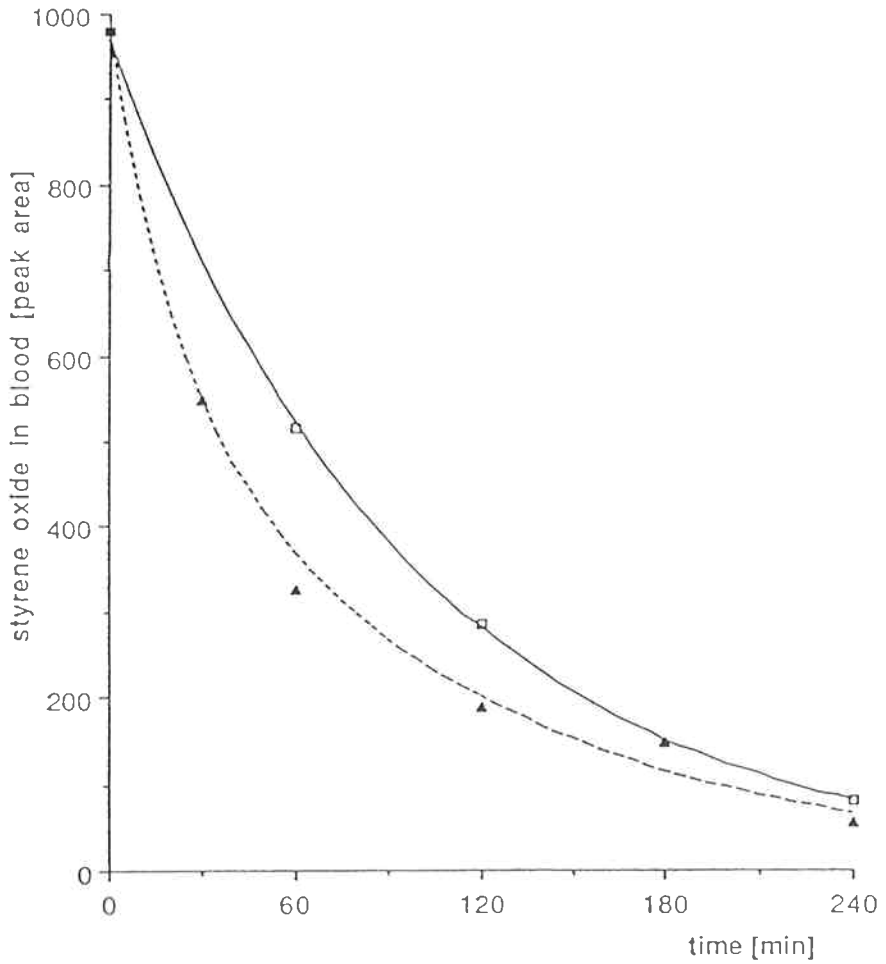


Cp1; V1: compartment 1 = blood with volume 1
Cp2; V2: compartment 2 = tissue with volume 2
y1; y2: concentrations in Cp1 and Cp2
k12; k21: microconstants of the transport between the phases
kel: rate constant of the elimination

FIGURE 2

Determination of the partition coefficients "tissue/blood" of styrene-7,8-oxide; Section 4.2.

Example: "fat/blood" of rats

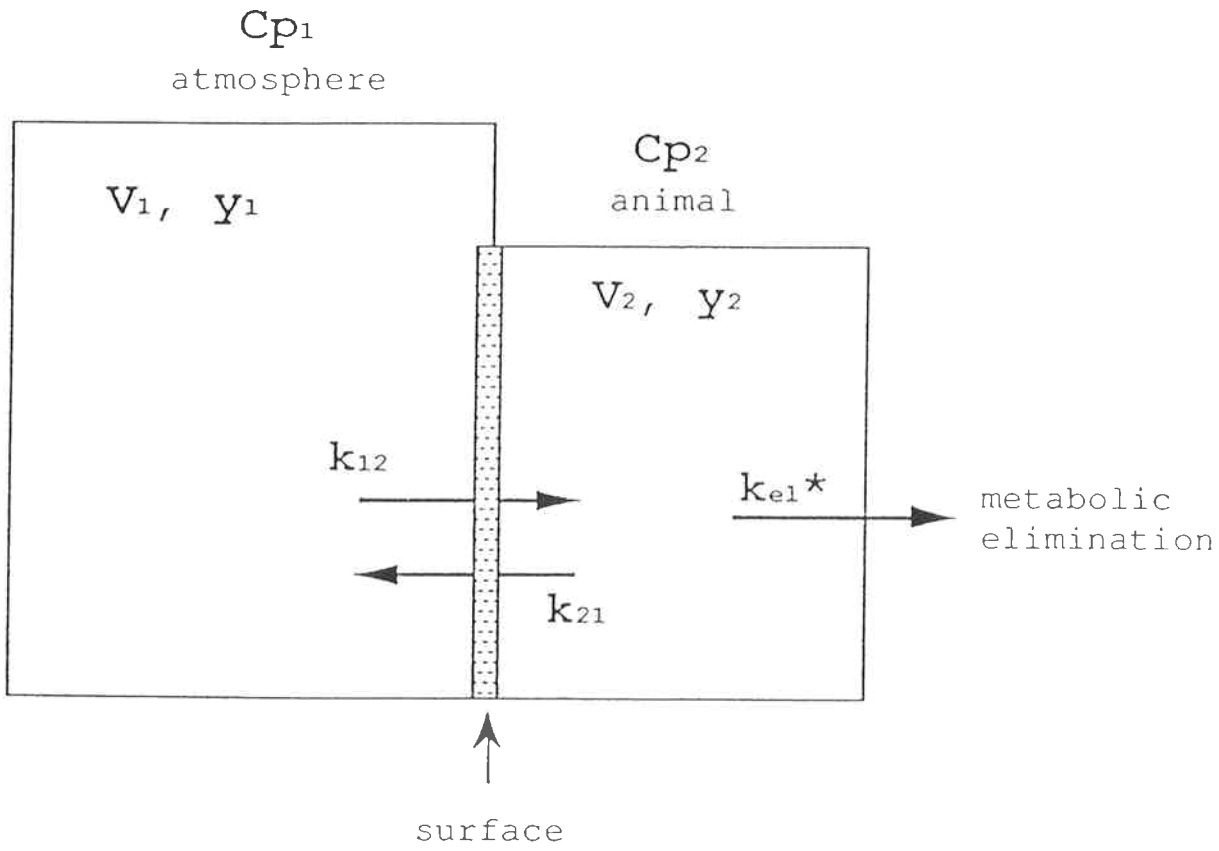


Concentration-time courses of styrene oxide incubated in vitro in blood (1 ml) and in blood (1 ml) + fat (0.1 g).

□ measured values in blood
▲ measured values in blood + fat
—— analyzed by 1 e-function
----- analyzed by 2 e-function

FIGURE 3

Pharmacokinetic two-compartment model for the closed exposure system; Section 4.2.

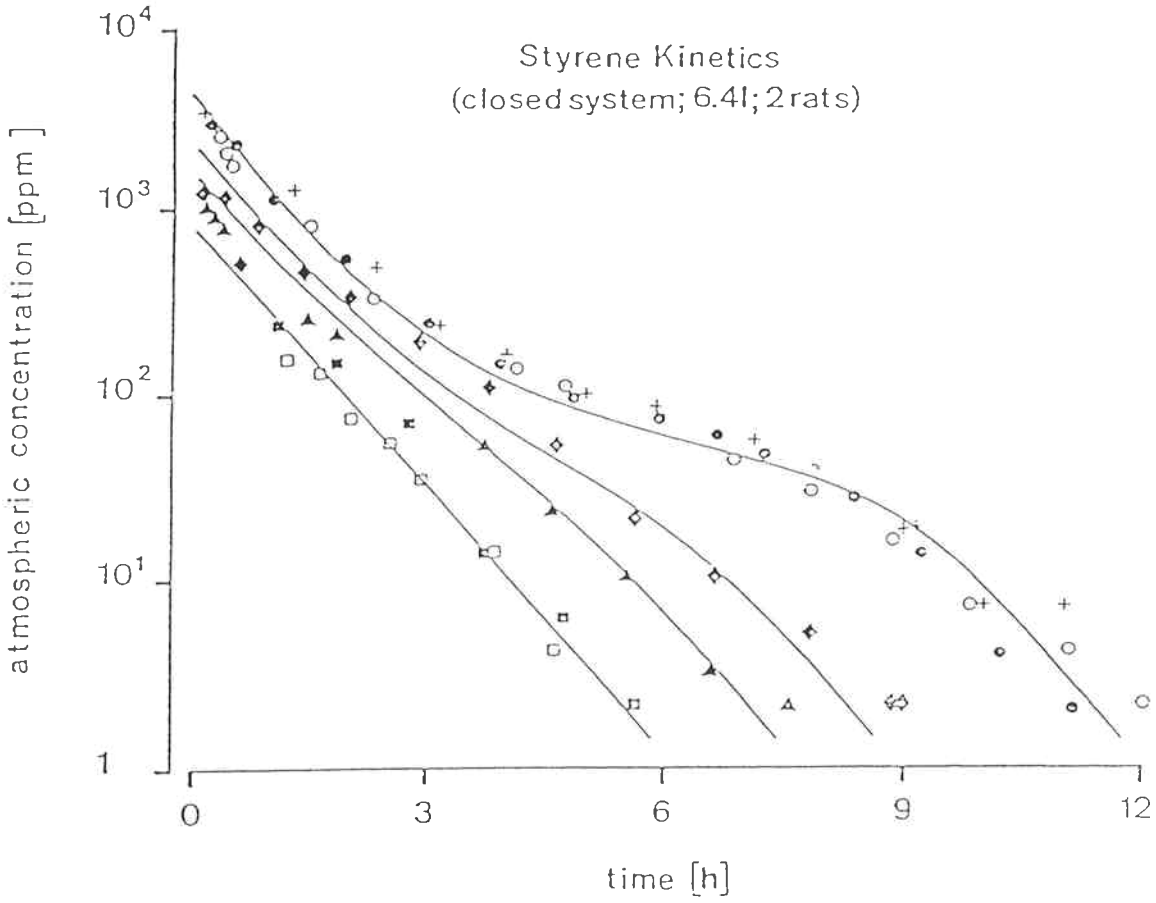


p_1; V_1: compartment 1 = atmosphere with volume 1
p_2; V_2: compartment 2 = animal with volume 2
1; y_2: concentrations in Cp_1 and Cp_2
12; k_{21}: microconstants of the inhalative uptake and exhalative elimination
el*: saturable metabolic elimination:

$$k_{el}^* = \frac{V_{max}}{V_2 \cdot (K_m + y_2)}$$

FIGURE 4

Inhalation kinetics of S; without pretreatment; rat
Section 4.3.

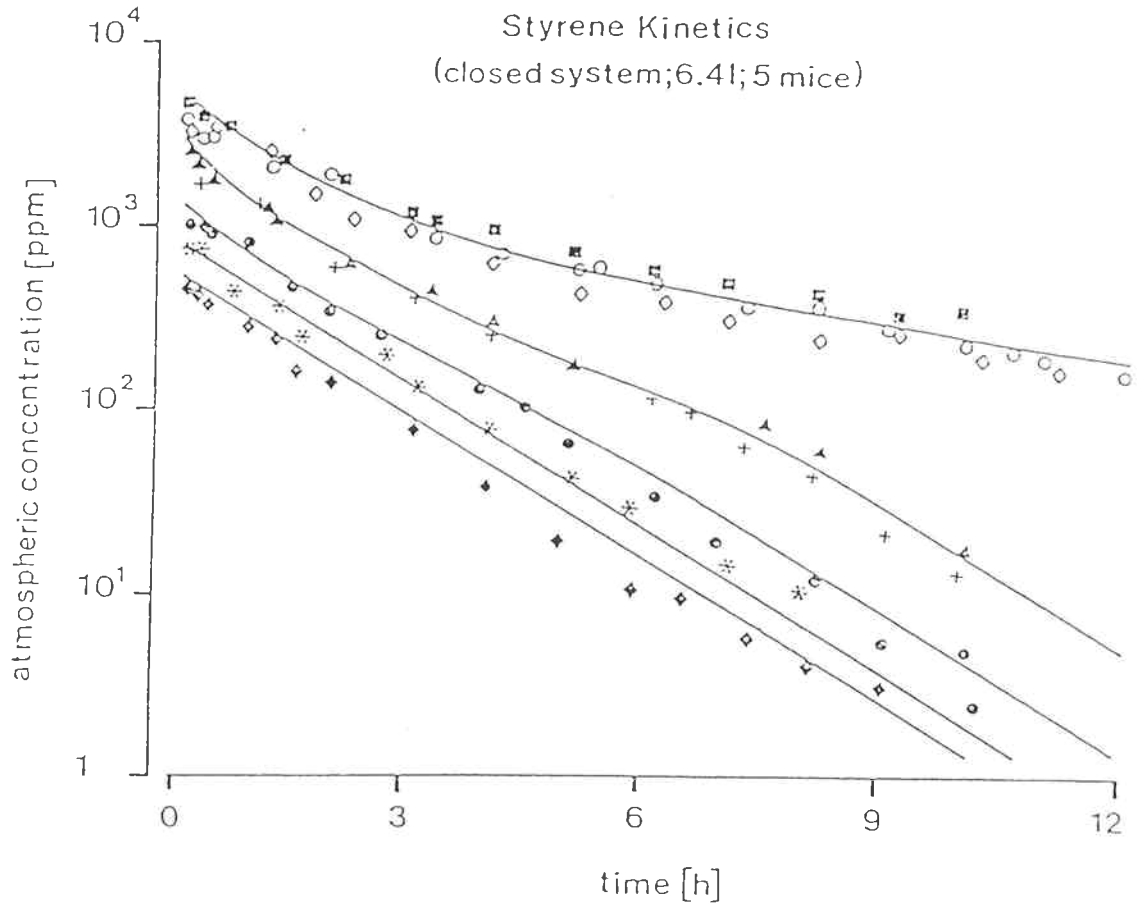


□ ■ Initial concentration 780 ppm; 2 experiments
▲ Initial concentration 1,600 ppm; 1 experiment
◆ Initial concentration 2,500 ppm; 1 experiment
○ ● + Initial concentration 3,800 ppm; 3 experiments

Curves are fitted using the pharmacokinetics model

FIGURE 5

Inhalation kinetics of S; without pretreatment; mouse
Section 4.3.



◆ Initial concentration 550 ppm; 1 experiment
✳ Initial concentration 800 ppm; 1 experiment
● Initial concentration 1,300 ppm; 1 experiment
+ ▲ Initial concentration 2,400 ppm; 2 experiments
■ ○ ◇ Initial concentration 5,000 ppm; 3 experiments

Curves are fitted using the pharmacokinetics model

FIGURE 6

Styrene kinetics; elimination; mouse, rat

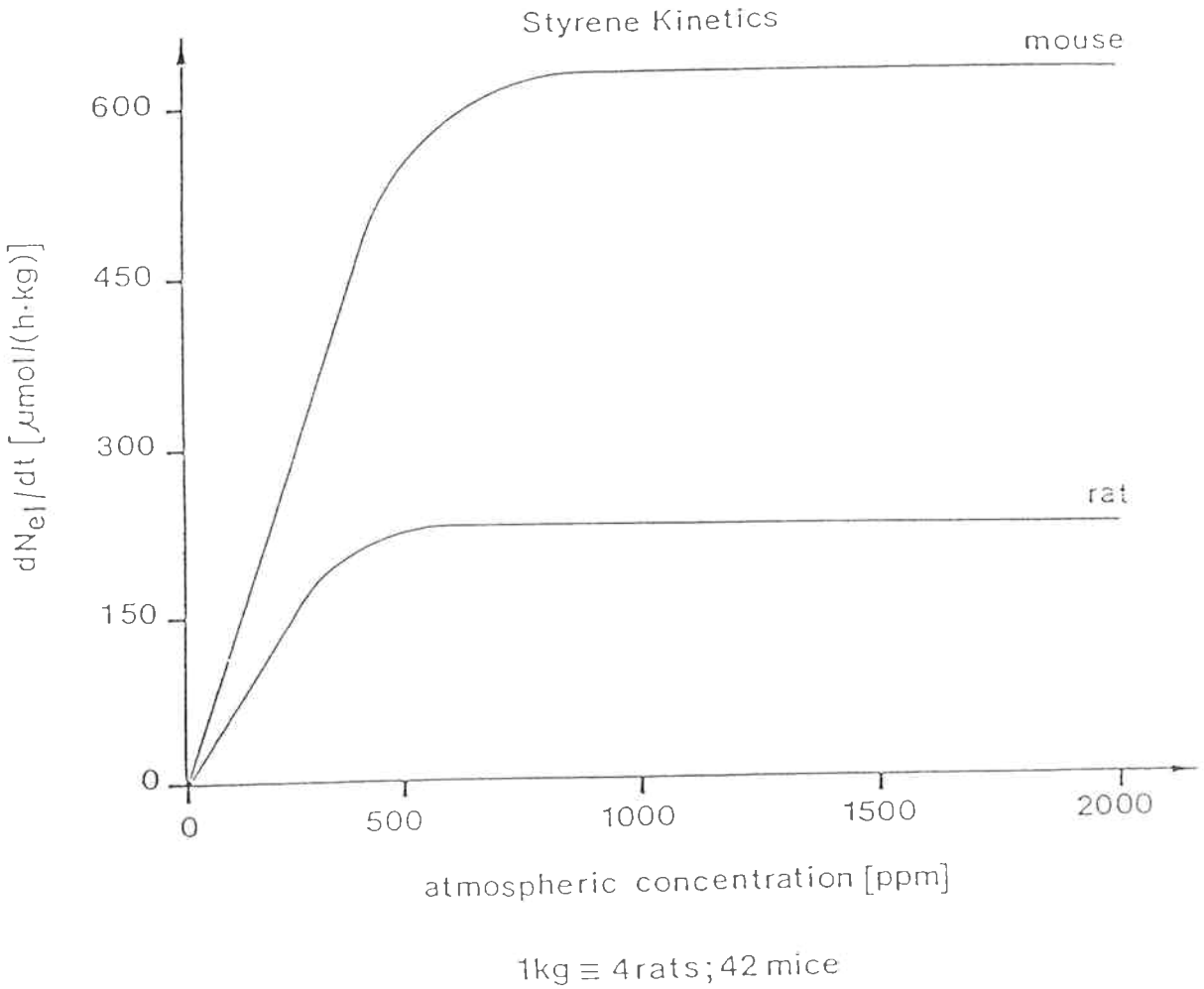


FIGURE 7

Styrene kinetics; bioaccumulation; mouse, rat.

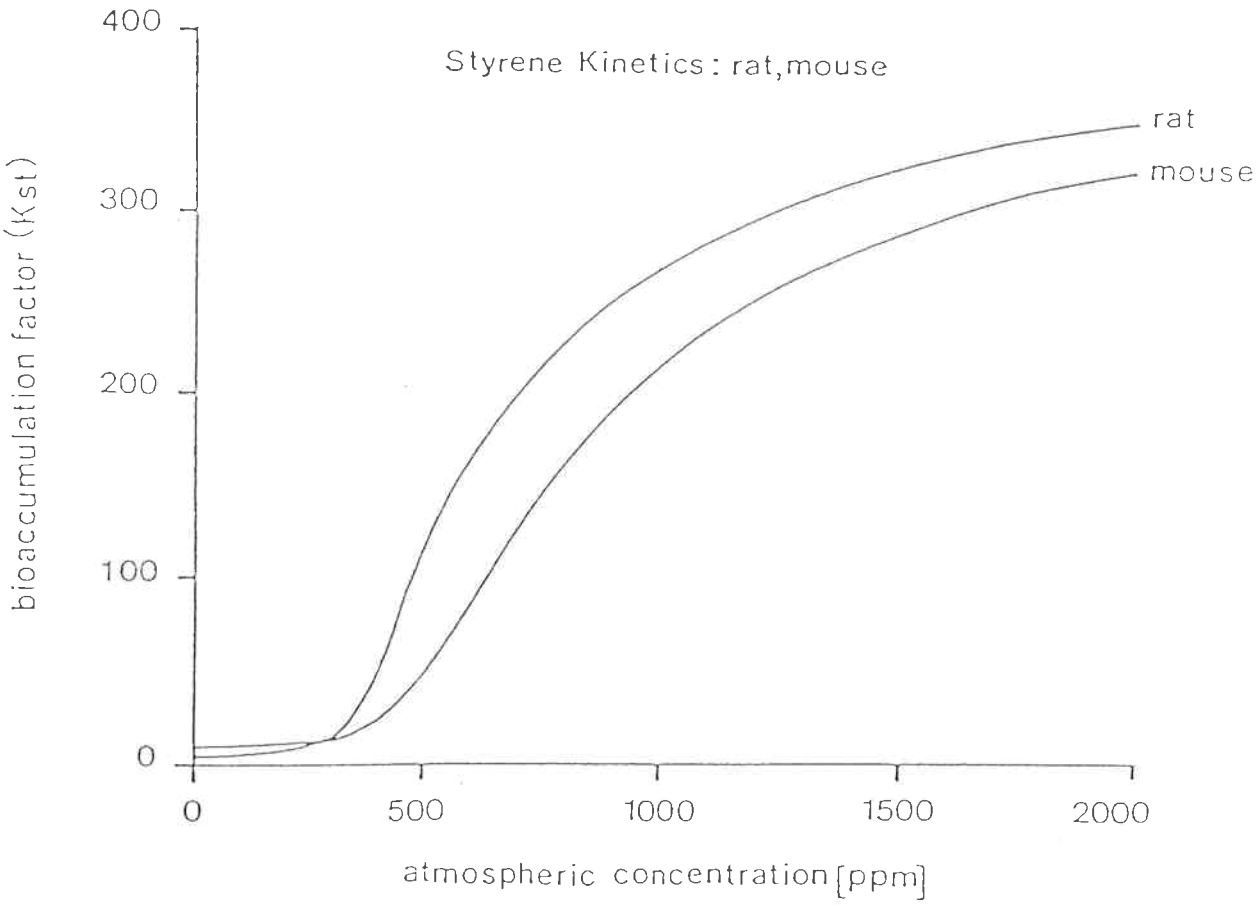


FIGURE 8

Styrene kinetics; concentration in the organism; mouse, rat

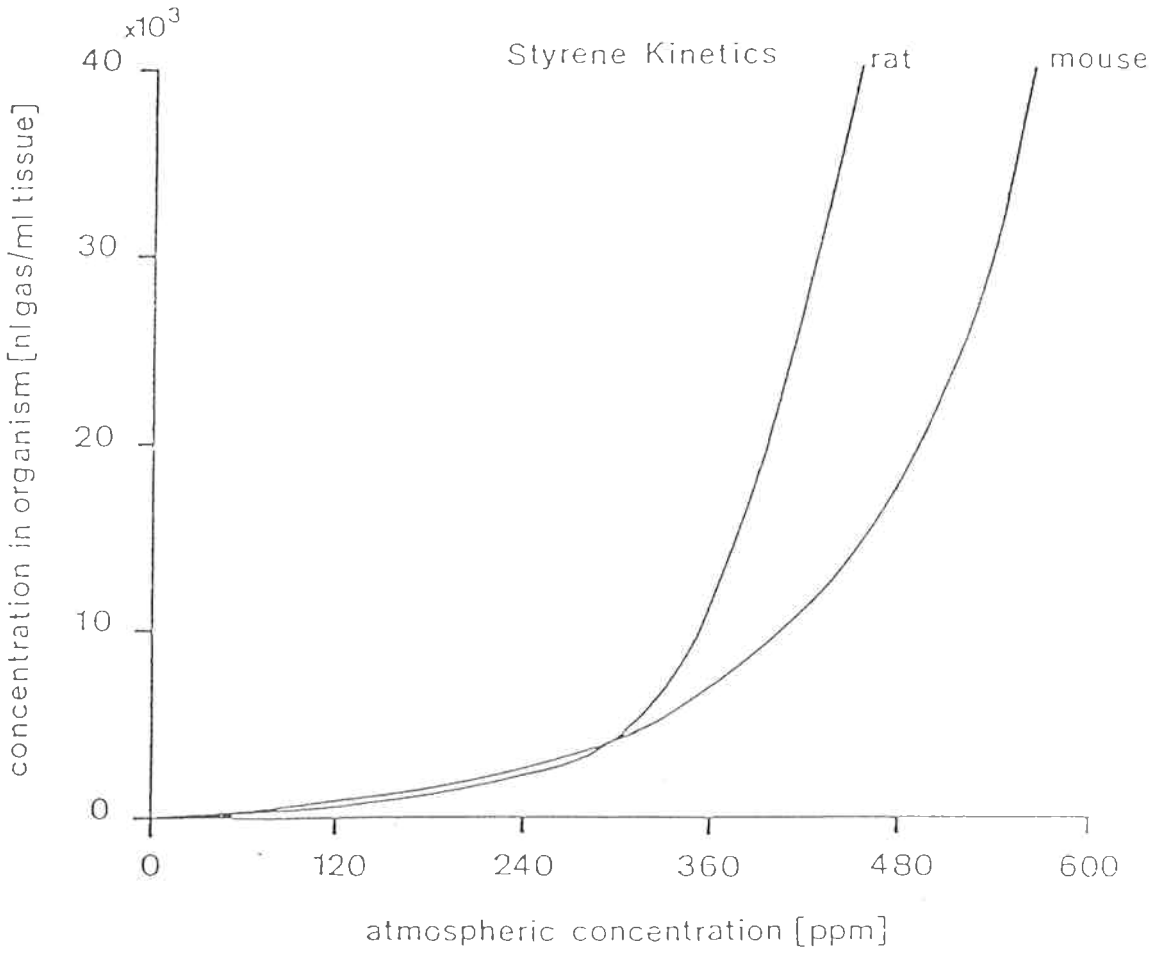
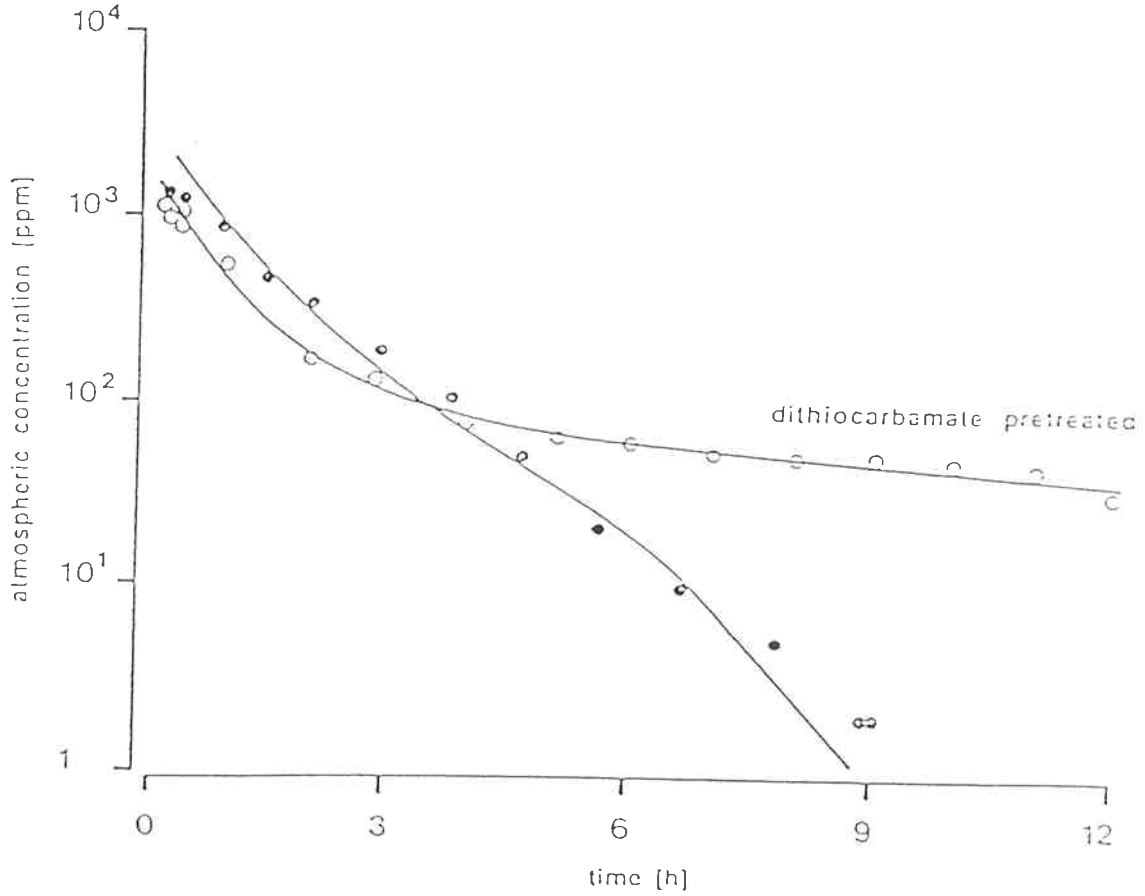


FIGURE 9

Inhalation kinetics of styrene; with dithiocarbamate pretreatment; rat
Section 4.3.

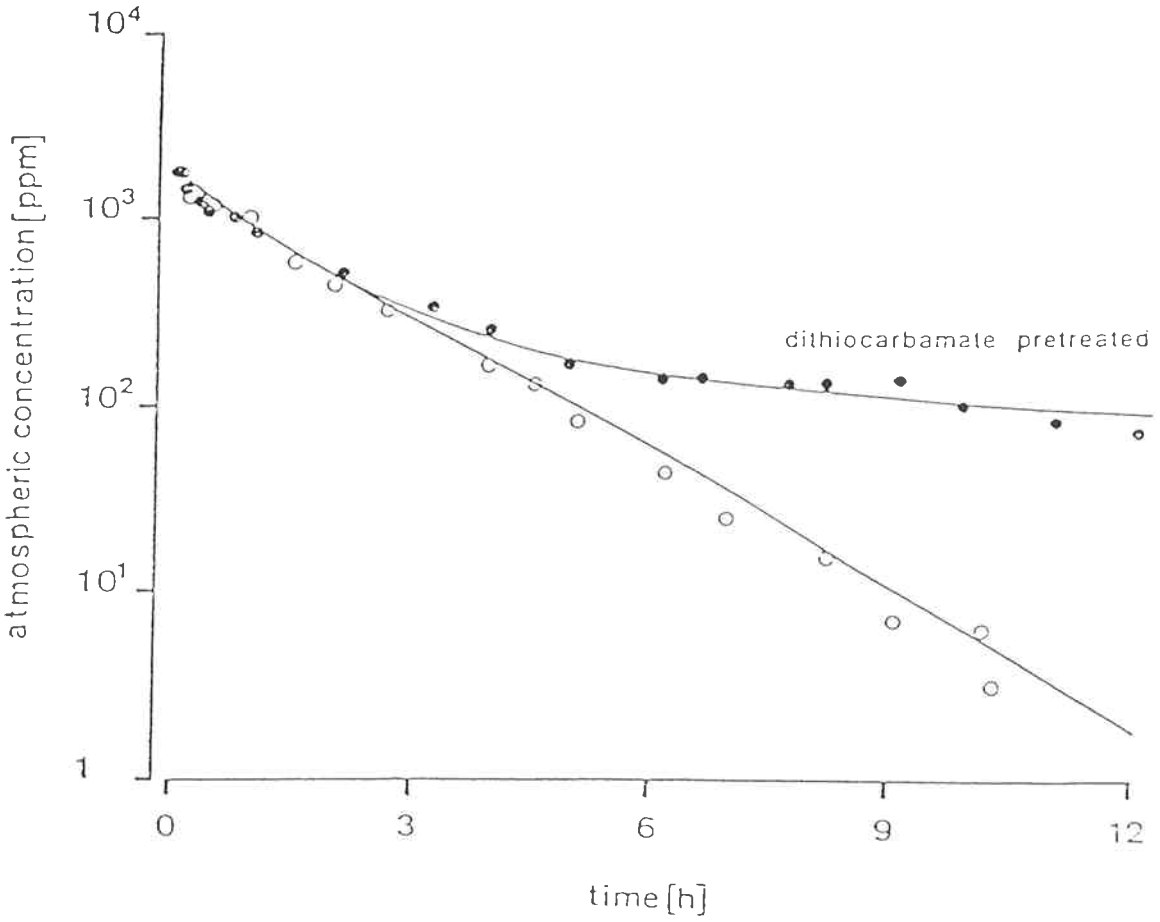


- O with pretreatment; initial concentration 1,400 ppm
- ● without pretreatment; initial concentration 2,500 ppm

Curves are fitted using the pharmacokinetic model

FIGURE 10

Inhalation kinetics of styrene; with dithiocarbamate pretreatment; mouse
Section 4.3.

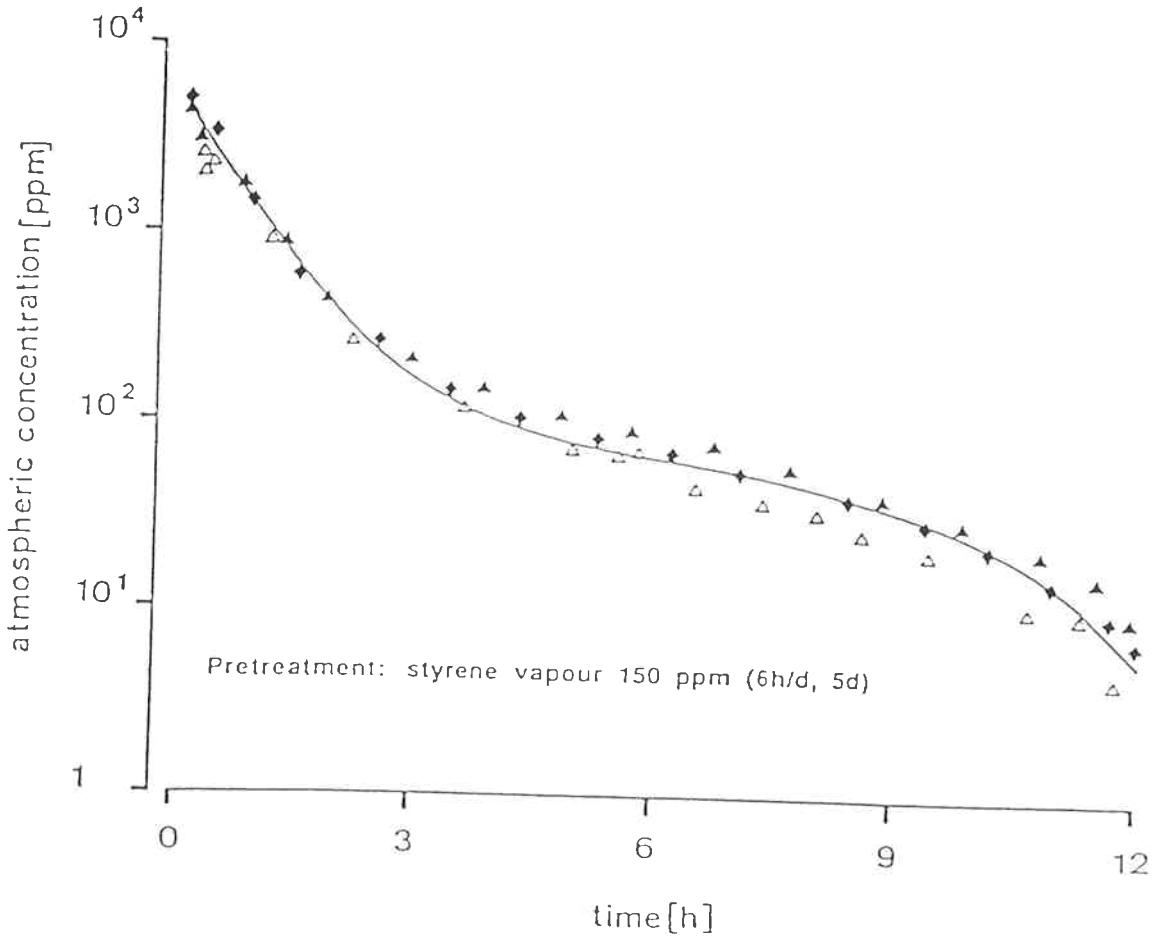


● with pretreatment; initial concentration 1,800 ppm
○ without pretreatment; initial concentration 1,600 ppm

Curves are fitted using the pharmacokinetic model

FIGURE 11

Inhalation kinetics of styrene; with pretreatment (repeated S inhalation); rat
Section 4.3.

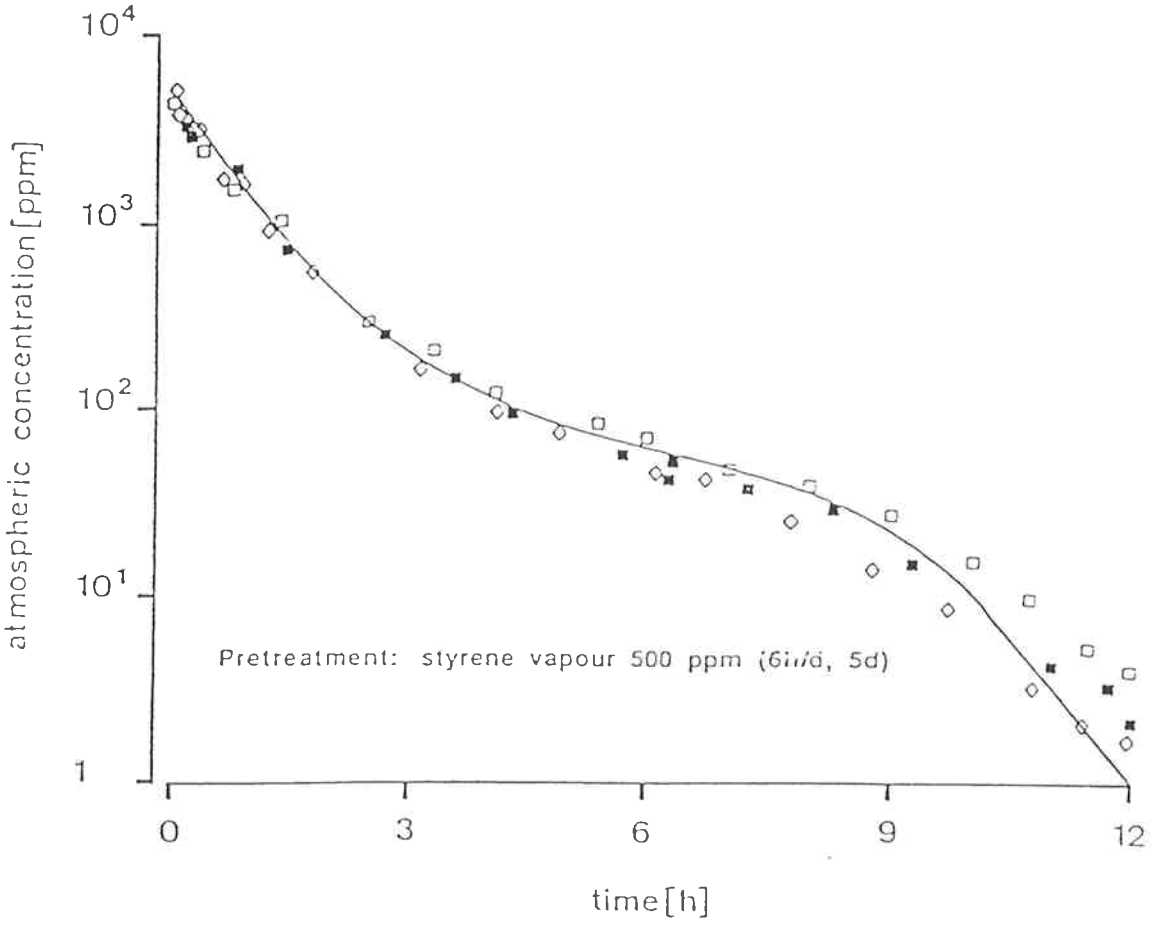


△ ◇ ▲ Initial concentration 4,700 ppm; 3 experiments

Curve predicted using pharmacokinetic model and assuming no effect

FIGURE 12

Inhalation kinetics of styrene; with pretreatment; rat
Section 4.3.

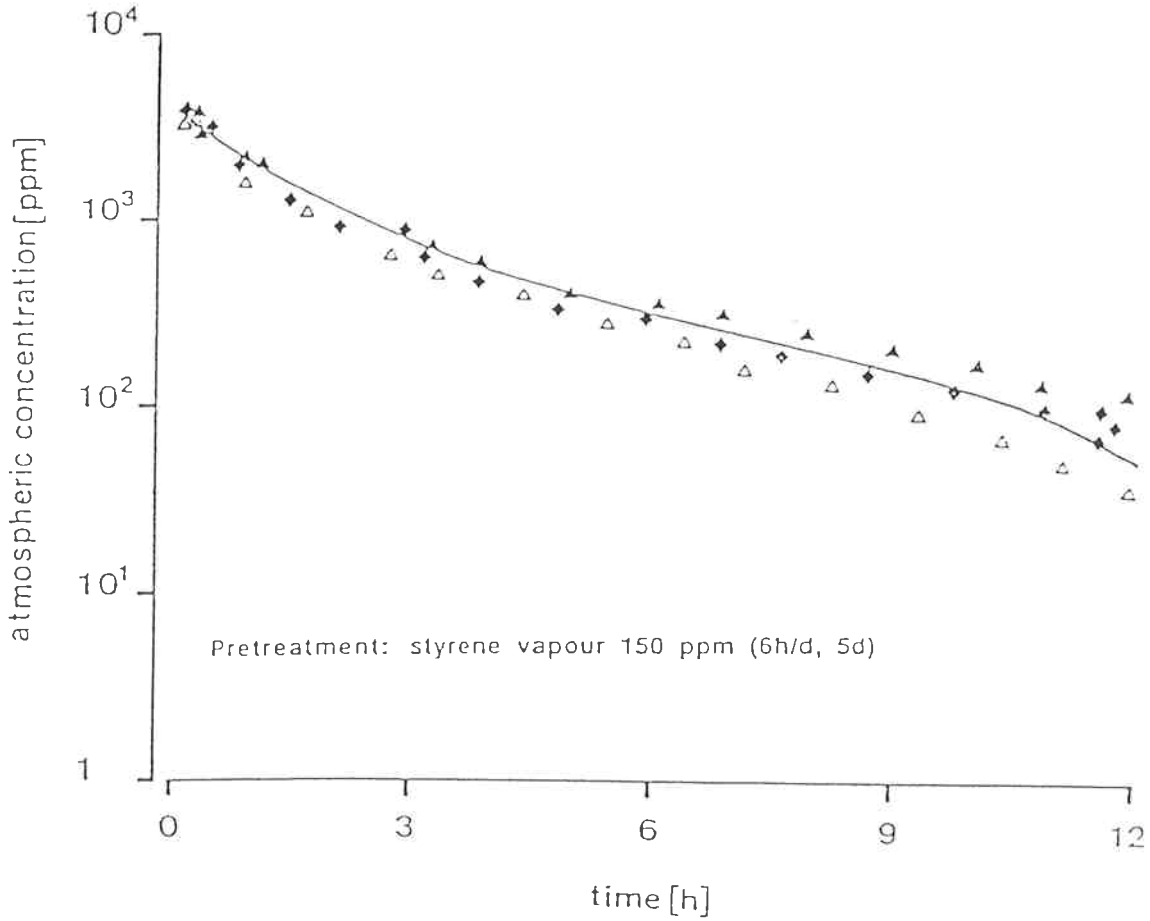


◇ ■ □ Initial concentration 4,200 ppm; 3 experiments

Curve predicted using pharmacokinetic model and assuming no effect

FIGURE 13

Inhalation kinetics of styrene; with pretreatment (repeated S inhalation); mouse
Section 4.3.

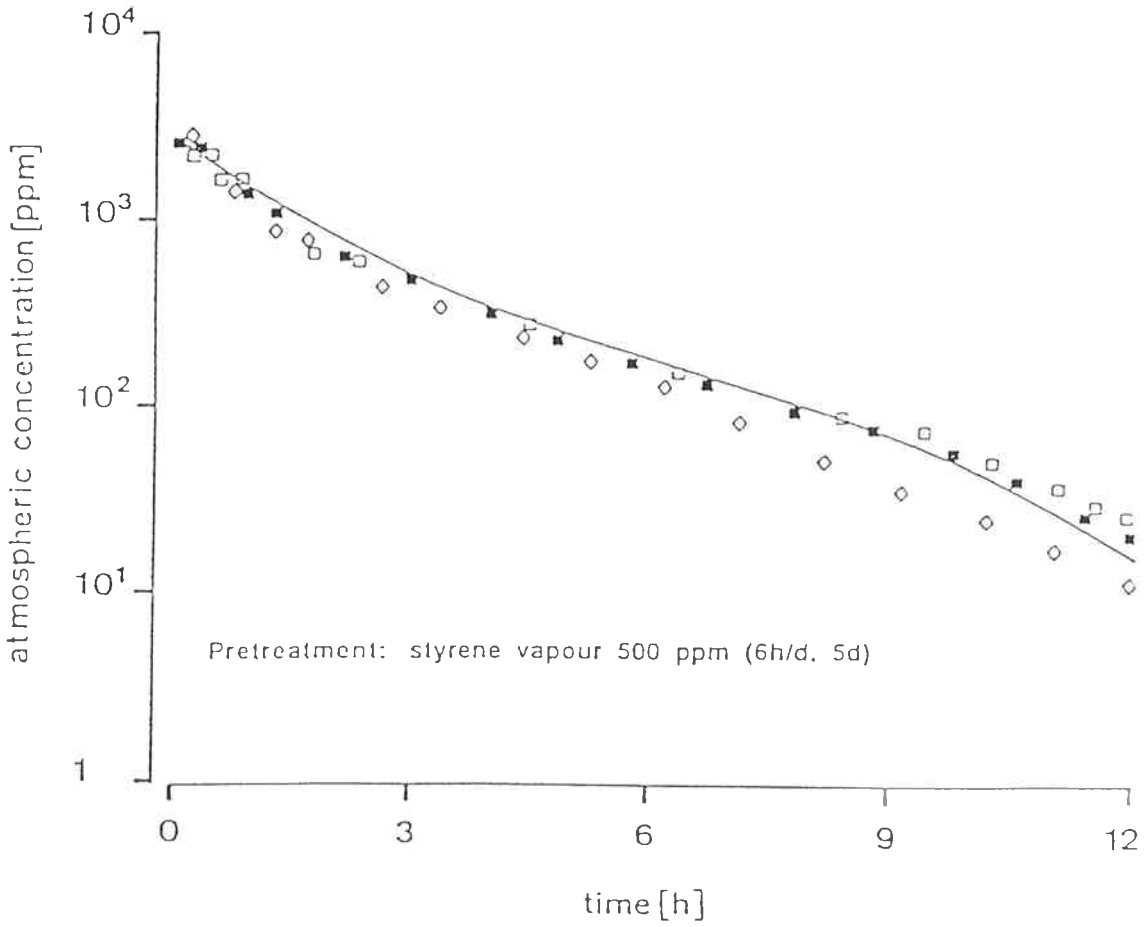


◆ △ ▲ Initial concentration 3,400 ppm; 3 experiments

Curve predicted using pharmacokinetic model and assuming no effect

FIGURE 14

Inhalation kinetics of styrene; with pretreatment (repeated S inhalation); mouse
Section 4.3.

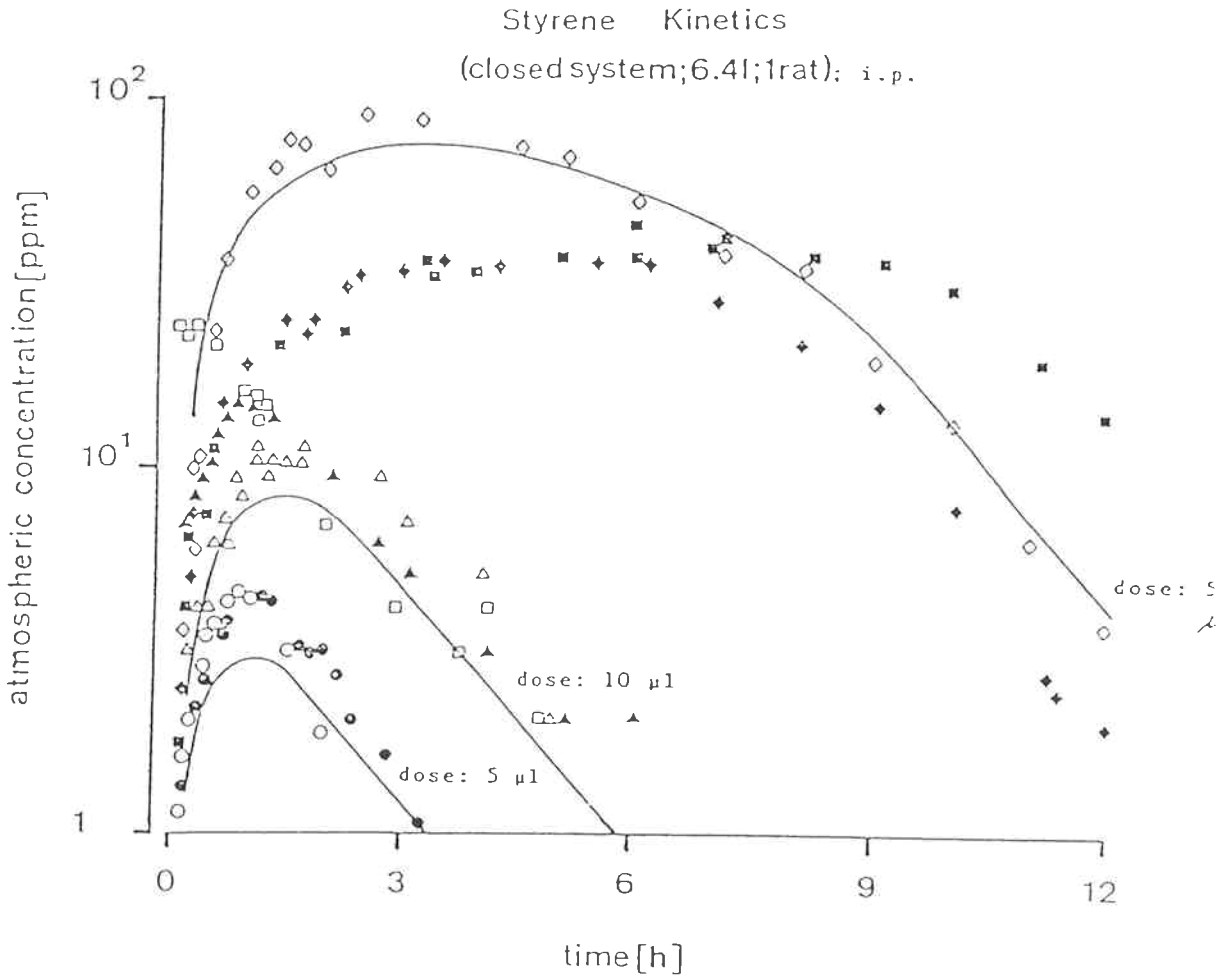


◇ ■ □ Initial concentration 2,600 ppm; 3 experiments

Curve predicted using pharmacokinetic model and assuming no effect

FIGURE 15

Kinetics of styrene after intraperitoneal application of styrene to rats; Section 4.4.



◆ ◇ ■ dose 50 µl S/animal; 3 experiments

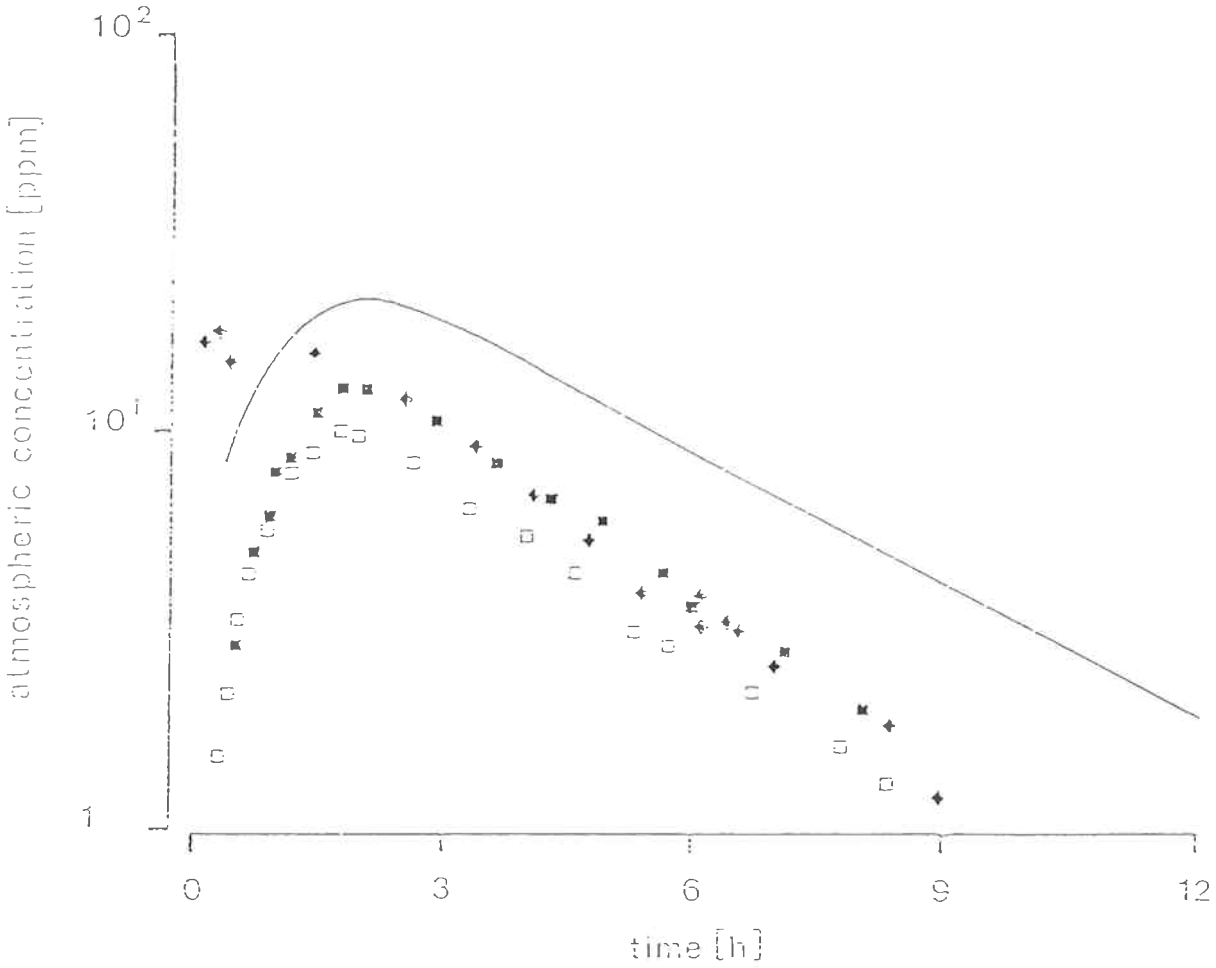
△ ▲ □ dose 10 µl S/animal; 3 experiments

○ ● dose 5 µl S/animal; 2 experiments

Curves are predicted from inhalation kinetics; equivalent doses

FIGURE 16

Kinetics of styrene after intraperitoneal application of styrene to mice; Section 4.4.

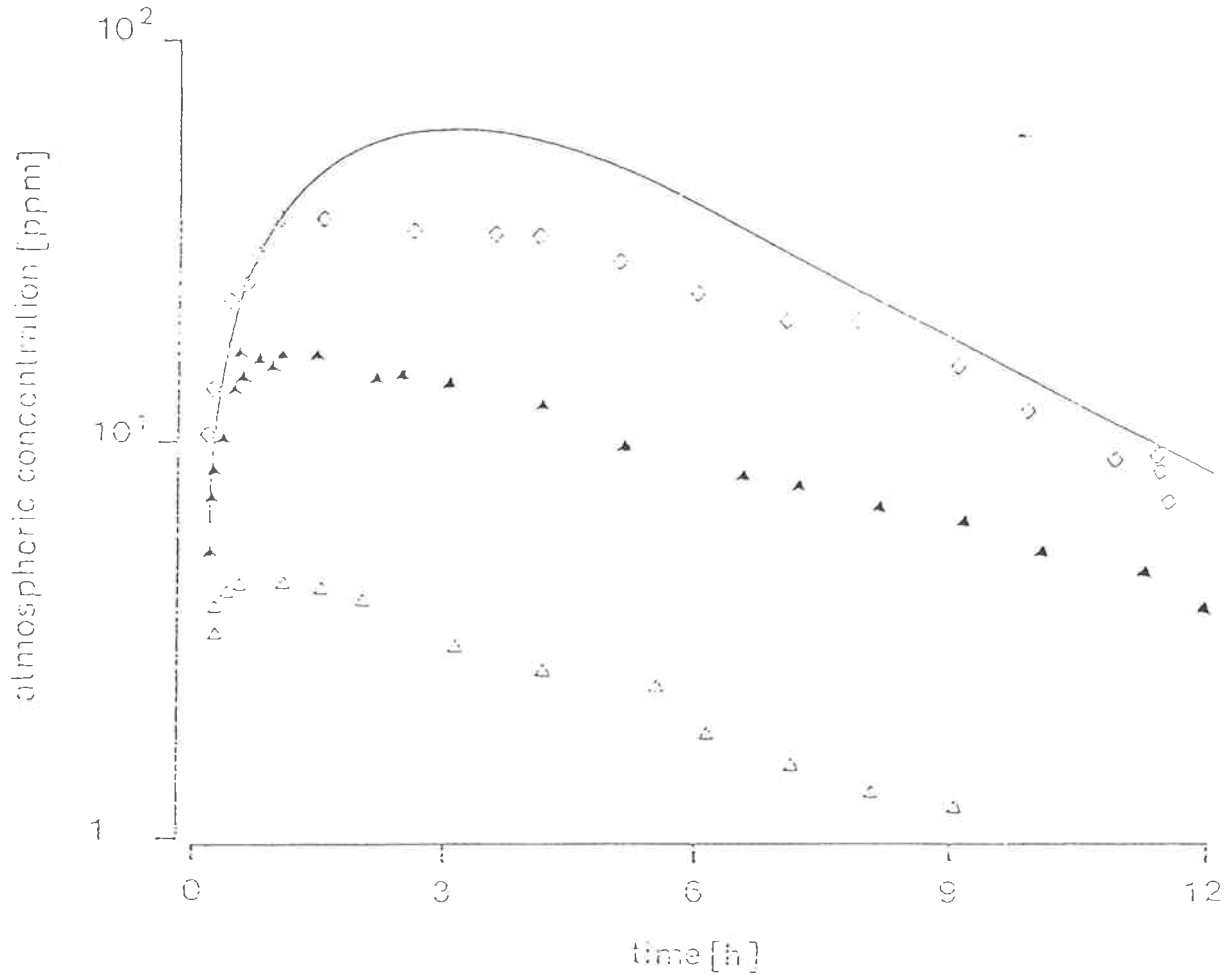


◆ □ ■ dose 5 µl S/animal; 3 experiments

Curve is predicted from inhalation kinetics; equivalent doses

FIGURE 17

Kinetics of styrene after intraperitoneal application of styrene to mice; Section 4.4.

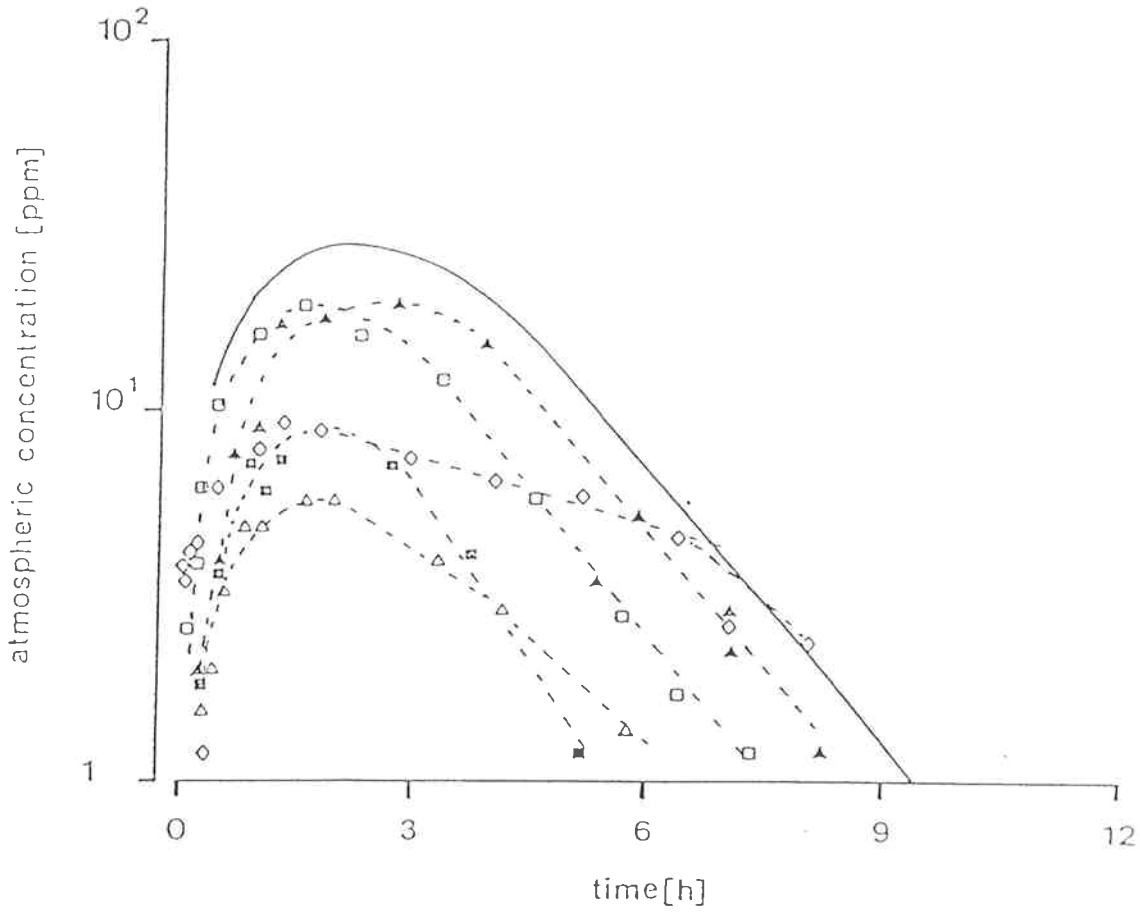


◇ ▲ △ dose 10 µl S/animal; 3 experiments

Curve is predicted from inhalation kinetics; equivalent doses

FIGURE 18

Kinetics of styrene after oral application of styrene to rats; Section 4.5.



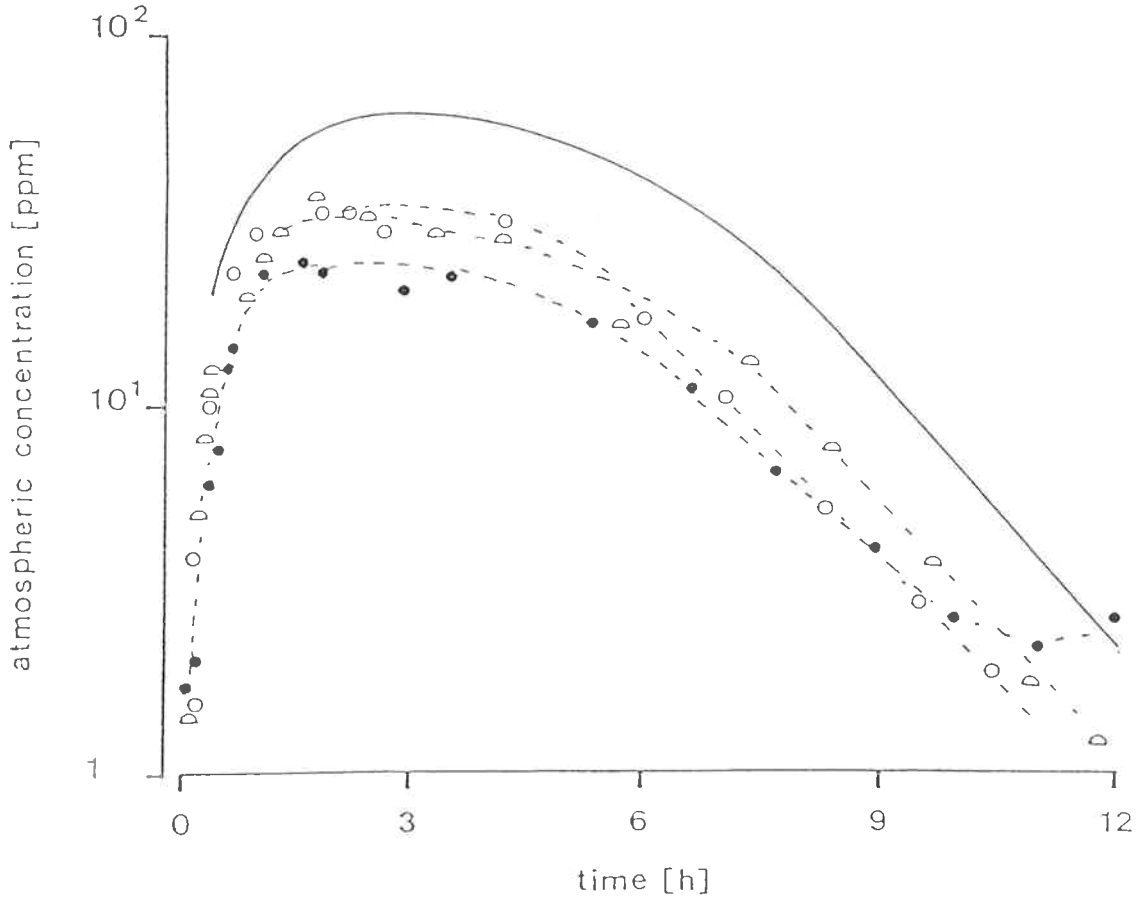
□ ■ ◇ △ ▲ dose 24 µl S in olive oil/animal; 5 experiments

------ curve represents one experiment

——— curve predicted from inhalation kinetics; equivalent dose

FIGURE 19

Kinetics of styrene after oral application of styrene to rats; Section 4.5.

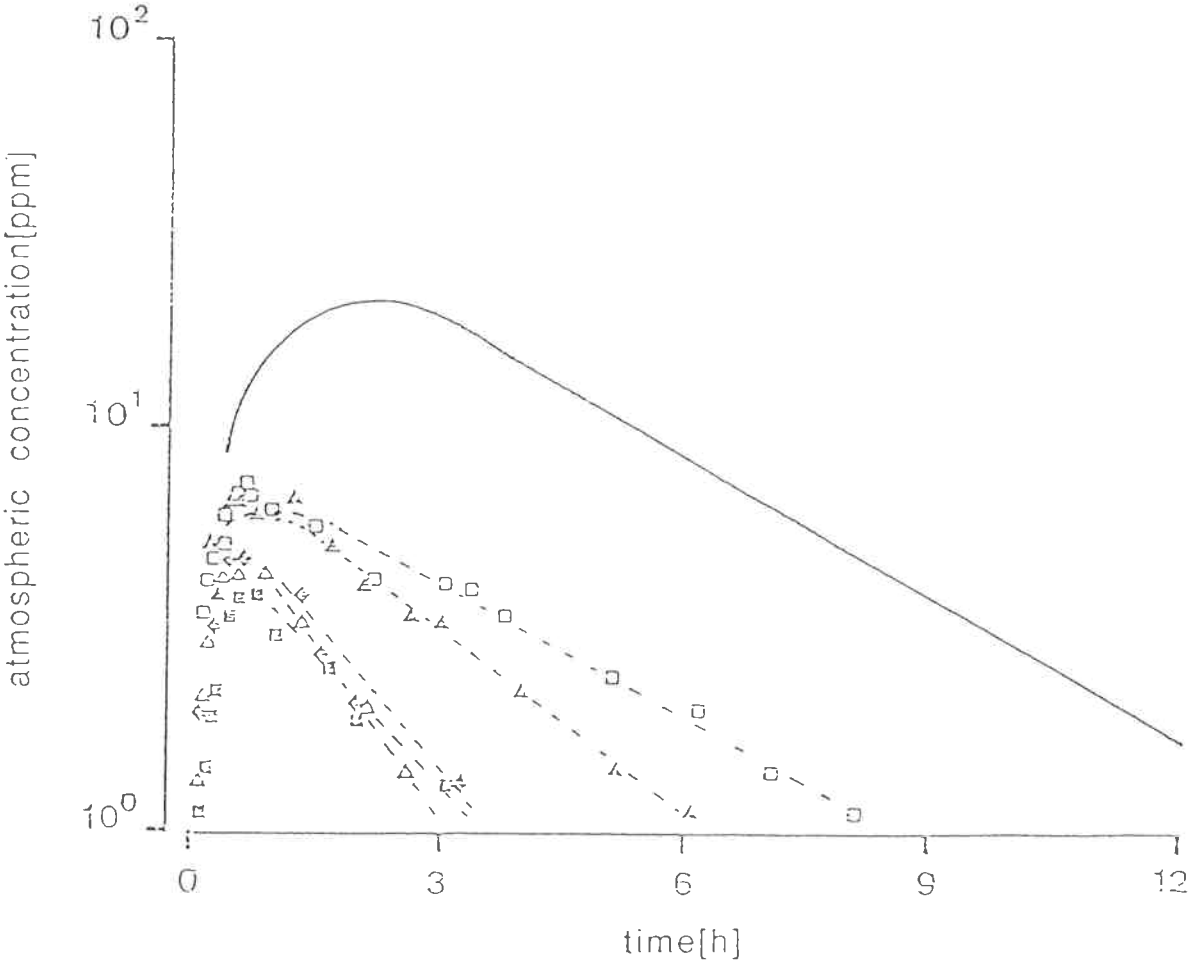


o △ ● dose 40 µl S in olive oil/animal; 3 experiments

——— Curve is predicted from inhalation kinetics; equivalent doses

FIGURE 20

Kinetics of styrene after oral application of styrene to mice; Section 4.5.



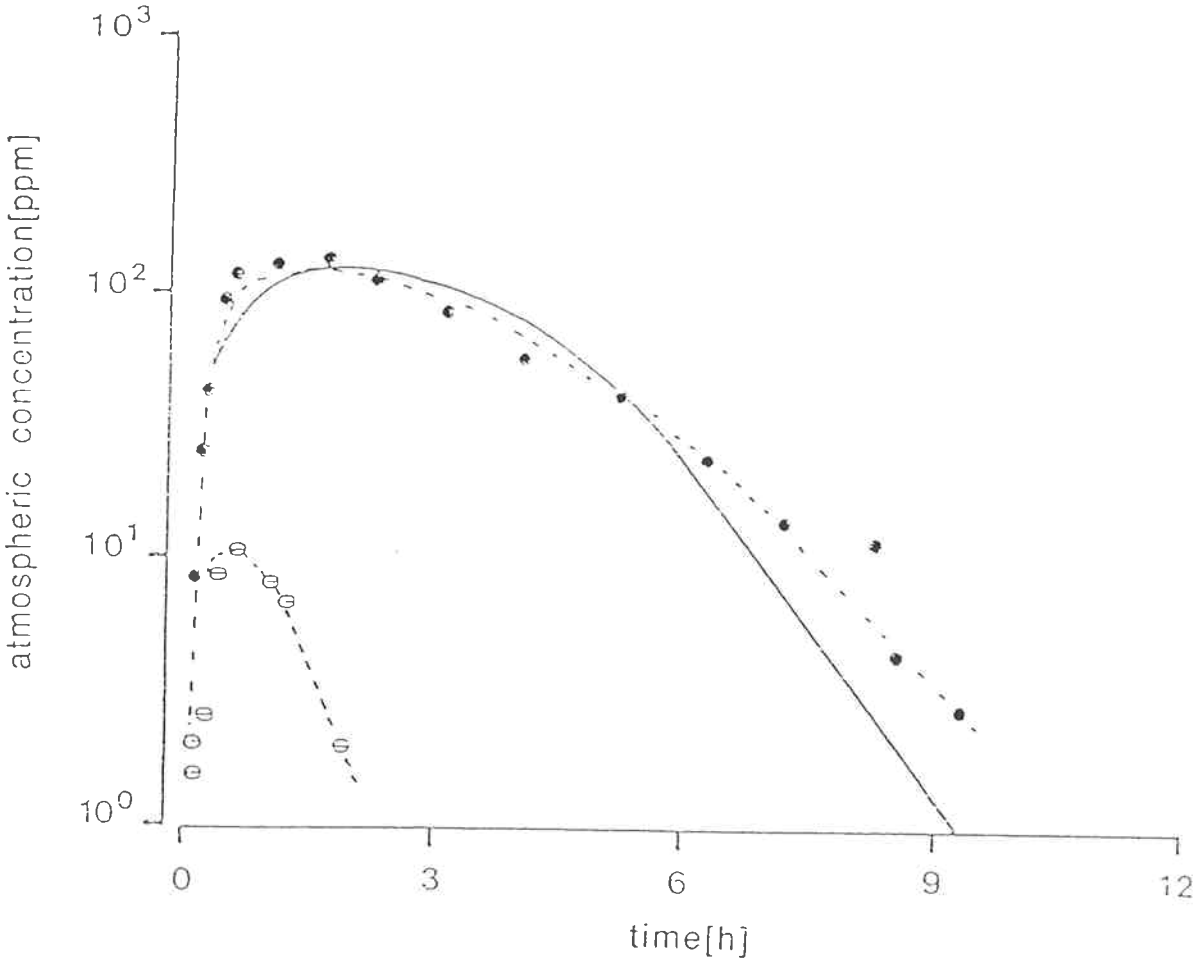
△ ▲ ◆ ■ □ dose 5 µl S in olive oil/animal; 5 experiments

------ curve represents one experiment

——— curve predicted from inhalation kinetics; equivalent dose

FIGURE 21

Kinetics of styrene after oral application of styrene to mice; Section 4.5.



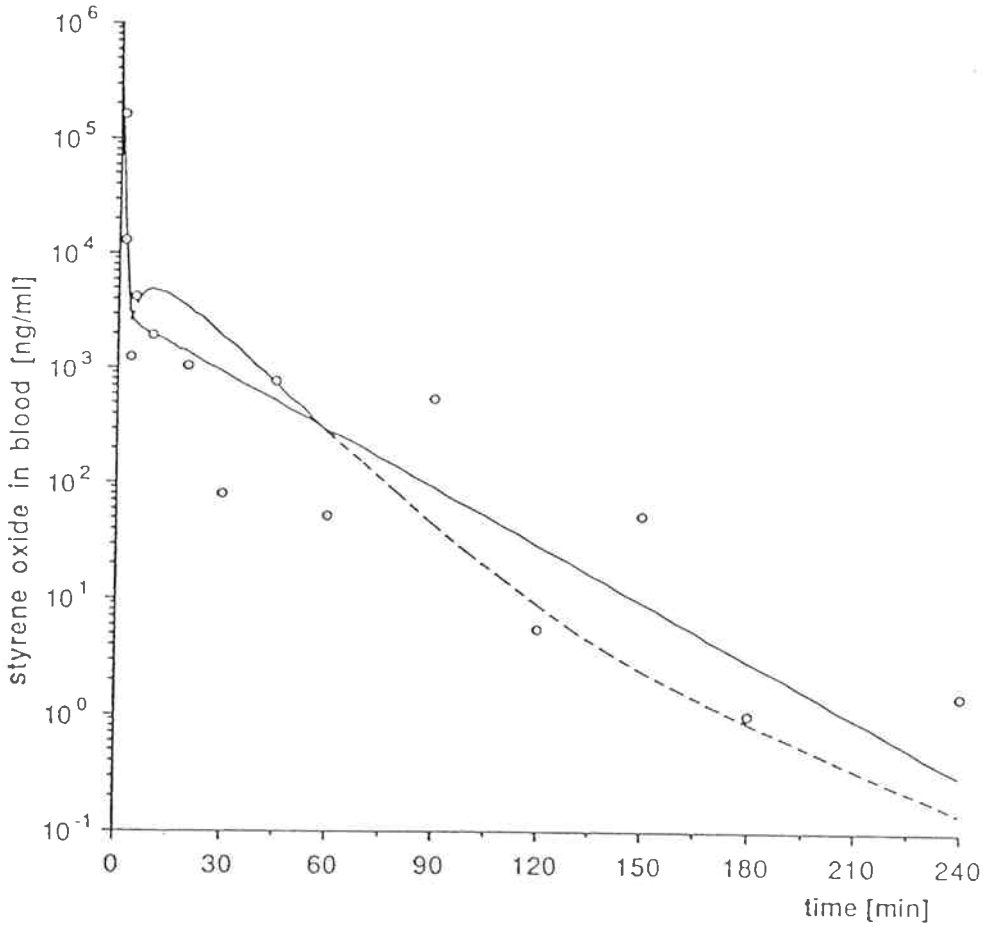
o ● dose 10 µl S in olive oil/animal; 2 experiments

- - - - - curve represents one experiment

——— curve predicted from inhalation kinetics; equivalent dose

FIGURE 22

Styrene-7,8-oxide in blood after intravenous administration of styrene oxide (25 mg/kg) to rats; Section 4.7.1.



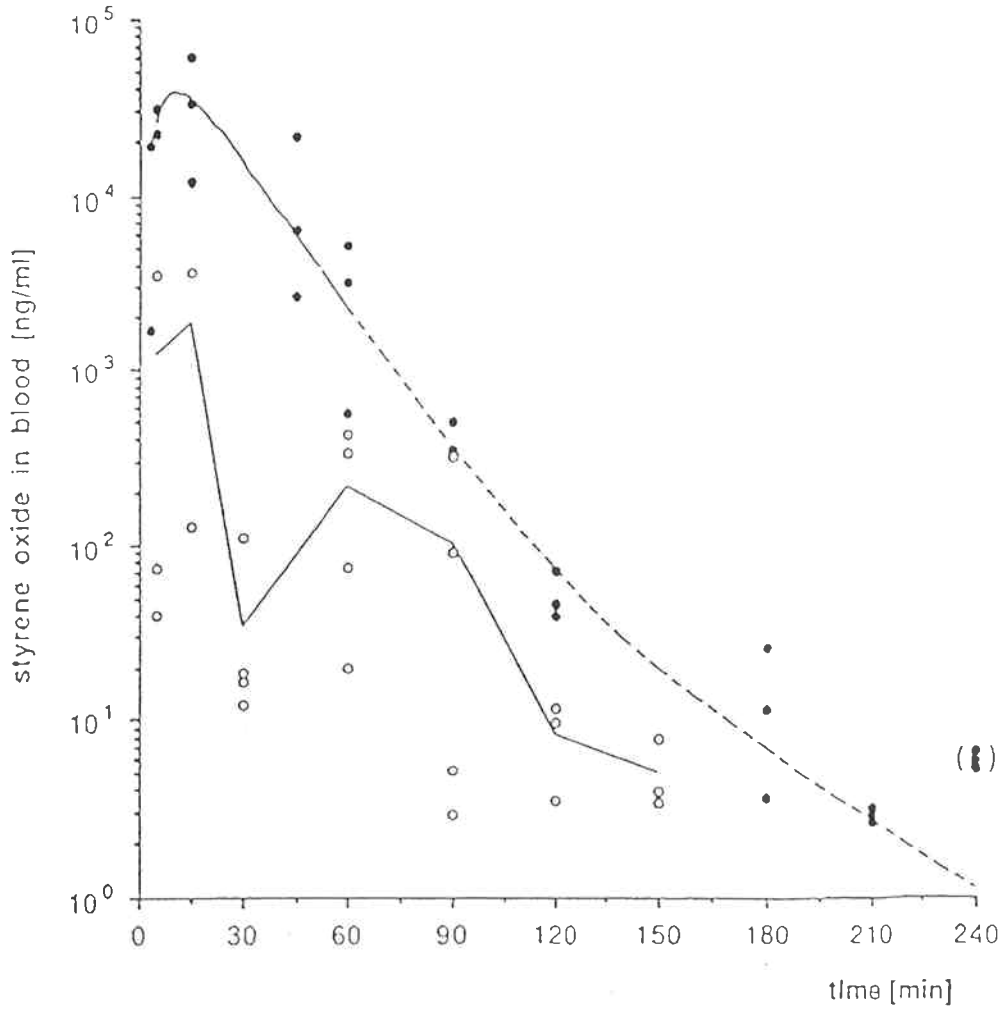
o measured values

——— fitted curve calculated with 2 e-function

– – – for comparison: calculated for i.p. administration of 25 mg SO/kg (obtained from the fitted curve in Fig. 23)

FIGURE 23

Styrene-7,8-oxide in blood after oral or intraperitoneal administration of styrene oxide to rats; Section 4.7.2. and 4.7.3.



o values after p.o. administration of 200 mg/kg; 4 experiments

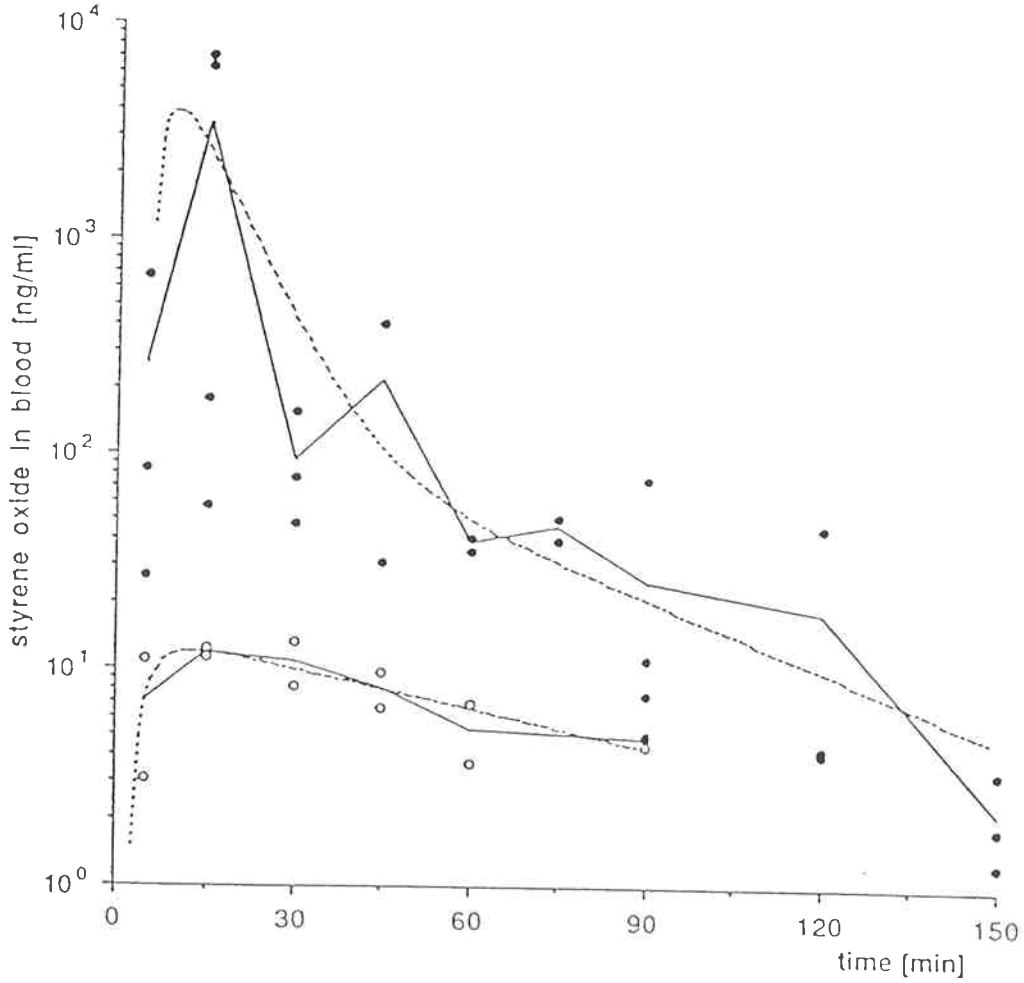
——— mean values

• values after i.p. administration of 200 mg/kg; 3 experiments

– – – fitted curve calculated with 3 e-functions

FIGURE 24

Styrene-7,8-oxide in blood after oral administration of styrene oxide to mice; Section 4.7.2.



o measured values after p.o. administration of 500 mg/kg; 2 experiments

——— mean values

• measured values after p.o. administration of 200 mg/kg; 3 experiments

– – – fitted curve calculated with 2 or 3 e-functions

FIGURE 25

Hydrolysis of styrene-7,8-oxide in aqueous systems (37 °C)

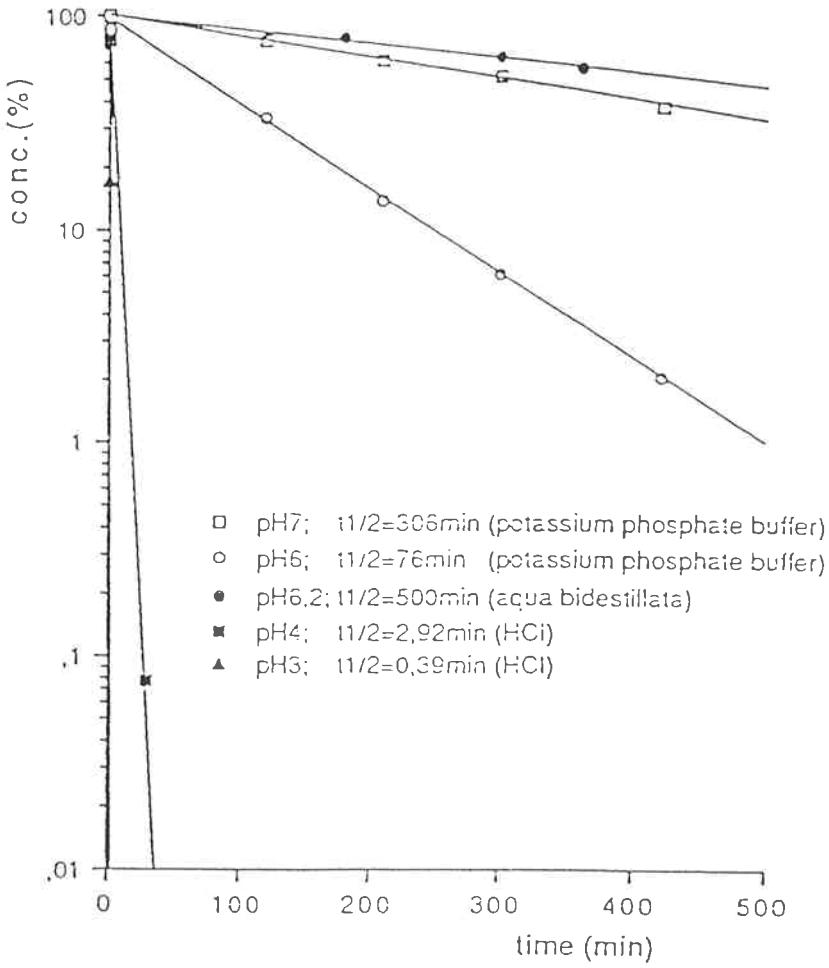


FIGURE 26

Half-lives of styrene-7,8-oxide in aqueous systems (37 °C)

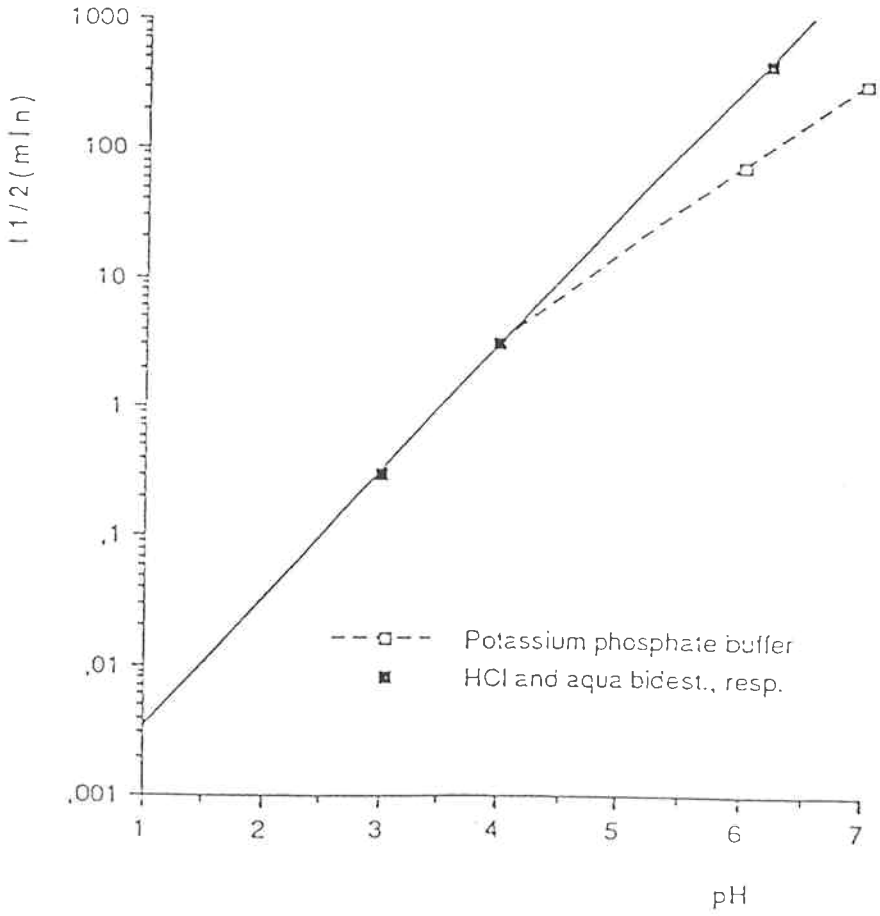
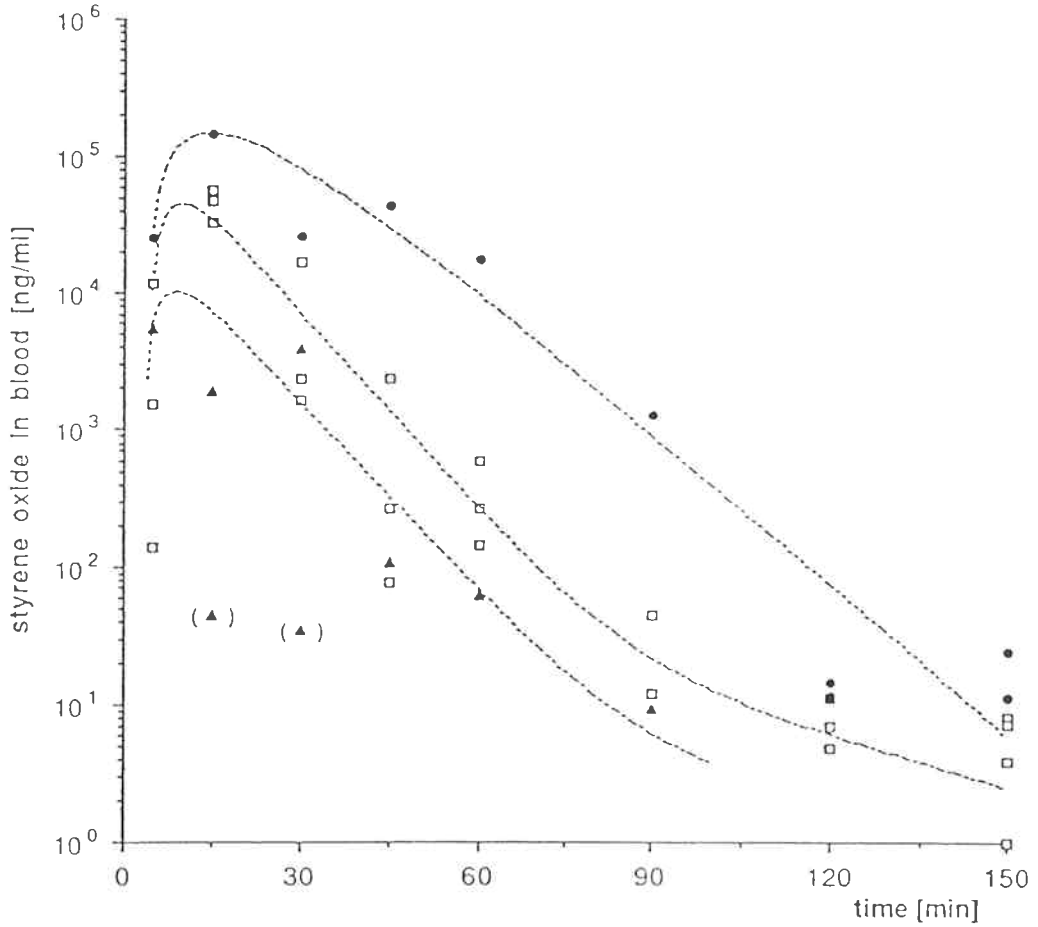


FIGURE 27

Styrene-7,8-oxide in blood after intraperitoneal administration of styrene oxide in mice; Section 4.7.3.



△ measured values after 100 mg/kg; 1 experiment

□ measured values after 200 mg/kg; 3 experiments

● measured values after 500 mg/kg; 1 experiment

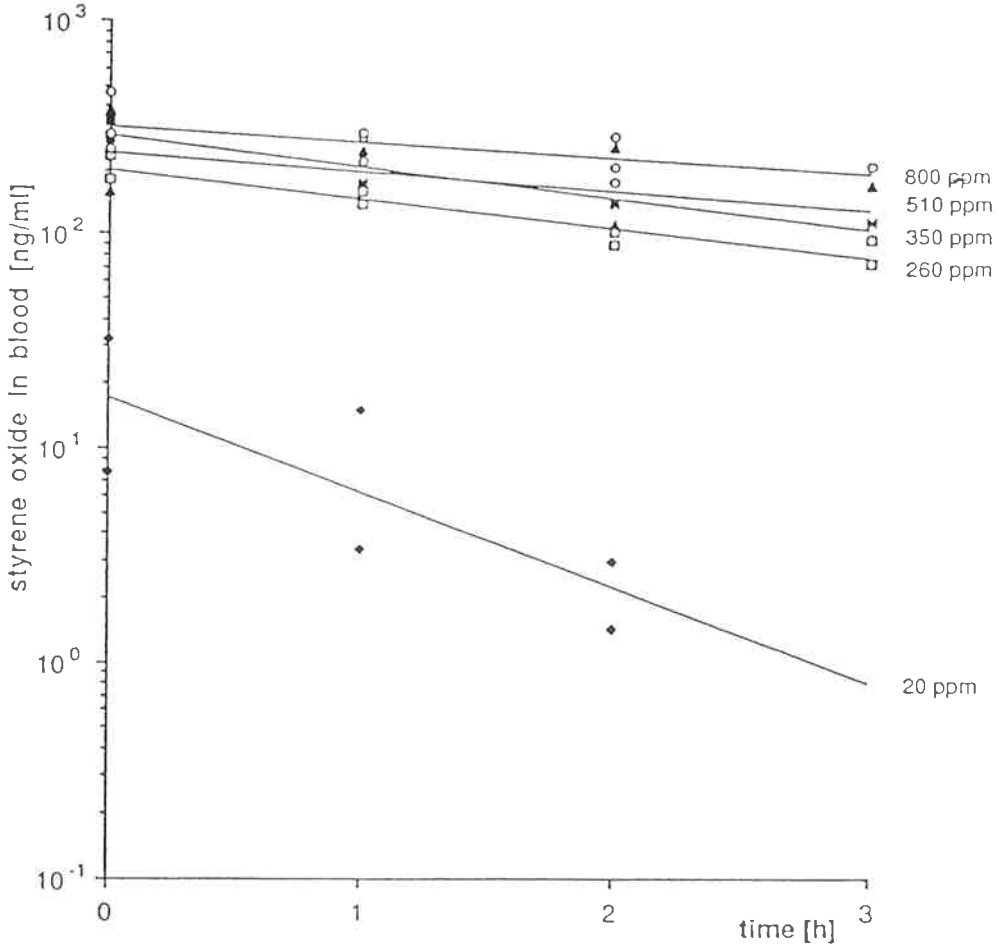
----- fitted curves calculated with 2 or 3 e-functions

point as outlier excluded

experiment repeated, duplicates only for 120 and 150 min

FIGURE 28

Styrene-7,8-oxide in blood after constant exposures to atmospheric styrene (steady-state conditions); rat
Section 4.7.4.



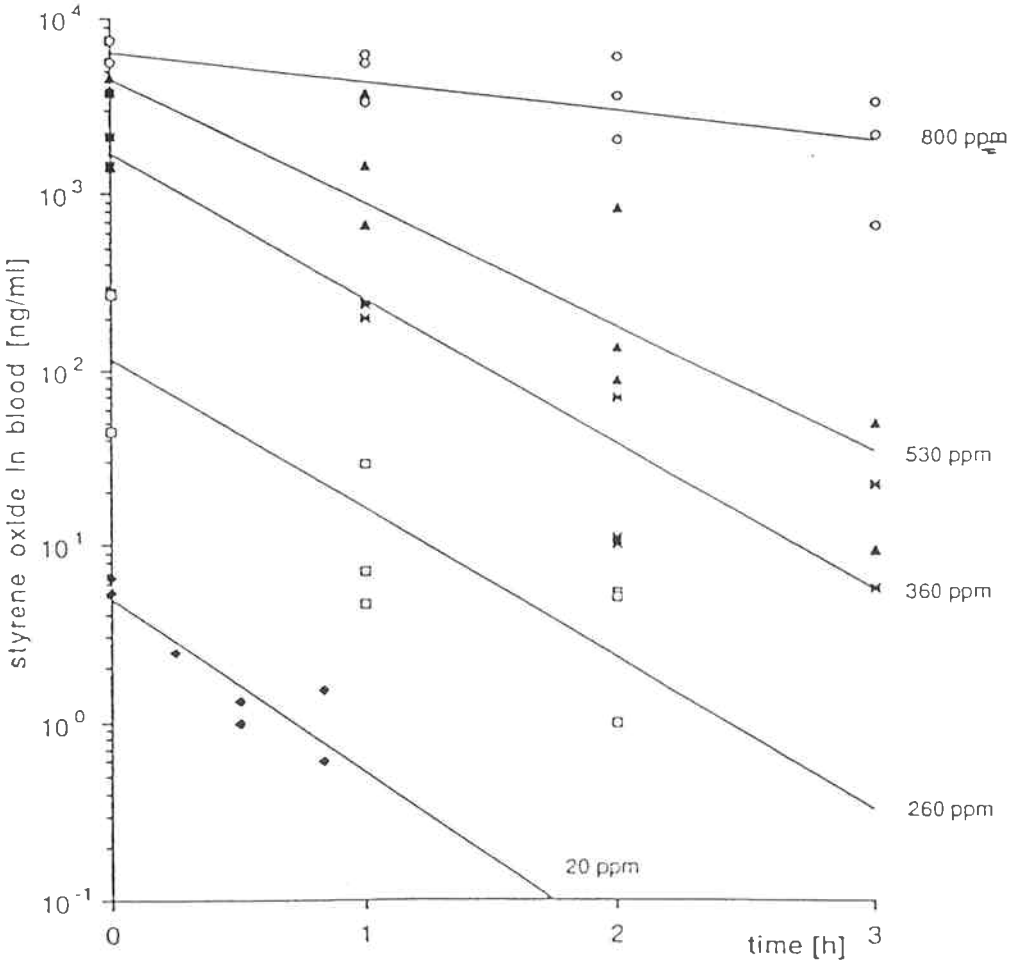
measured values after exposure to styrene at concentrations of

- ◆ 20 ppm (y= 17*10^(-0.446x), t1/2=0.7 h)
- □ 260 ppm (y=202*10^(-0.141x), t1/2=2.1 h)
- ✖ 350 ppm (y=291*10^(-0.154x), t1/2=2.0 h)
- ▲ 510 ppm (y=240*10^(-0.092x), t1/2=3.3 h)
- ○ 800 ppm (y=318*10^(-0.077x), t1/2=3.9 h)

formulas in brackets represent exponential regressions and resulting half-lives

FIGURE 29

Styrene-7,8-oxide in blood after constant exposures to atmospheric styrene (steady-state conditions); mouse
Section 4.7.4.



measured values after exposure to styrene at concentrations of

- ◆ 20 ppm (y= 4.9*10^(-0.973x), t1/2=0.31 h)
- □ 260 ppm (y= 116*10^(-0.848x), t1/2=0.35 h)
- ✖ 360 ppm (y=1691*10^(-0.828x), t1/2=0.36 h)
- ▲ 530 ppm (y=4537*10^(-0.711x), t1/2=0.42 h)
- ○ 800 ppm (y=6314*10^(-0.173x), t1/2=1.70 h)

formulas in brackets represent exponential regressions and resulting half-lives

FIGURE 30

Styrene-7,8-oxide in blood at the end of the 3-hour steady state S exposure period (see section 4.7.4.); rat, mouse

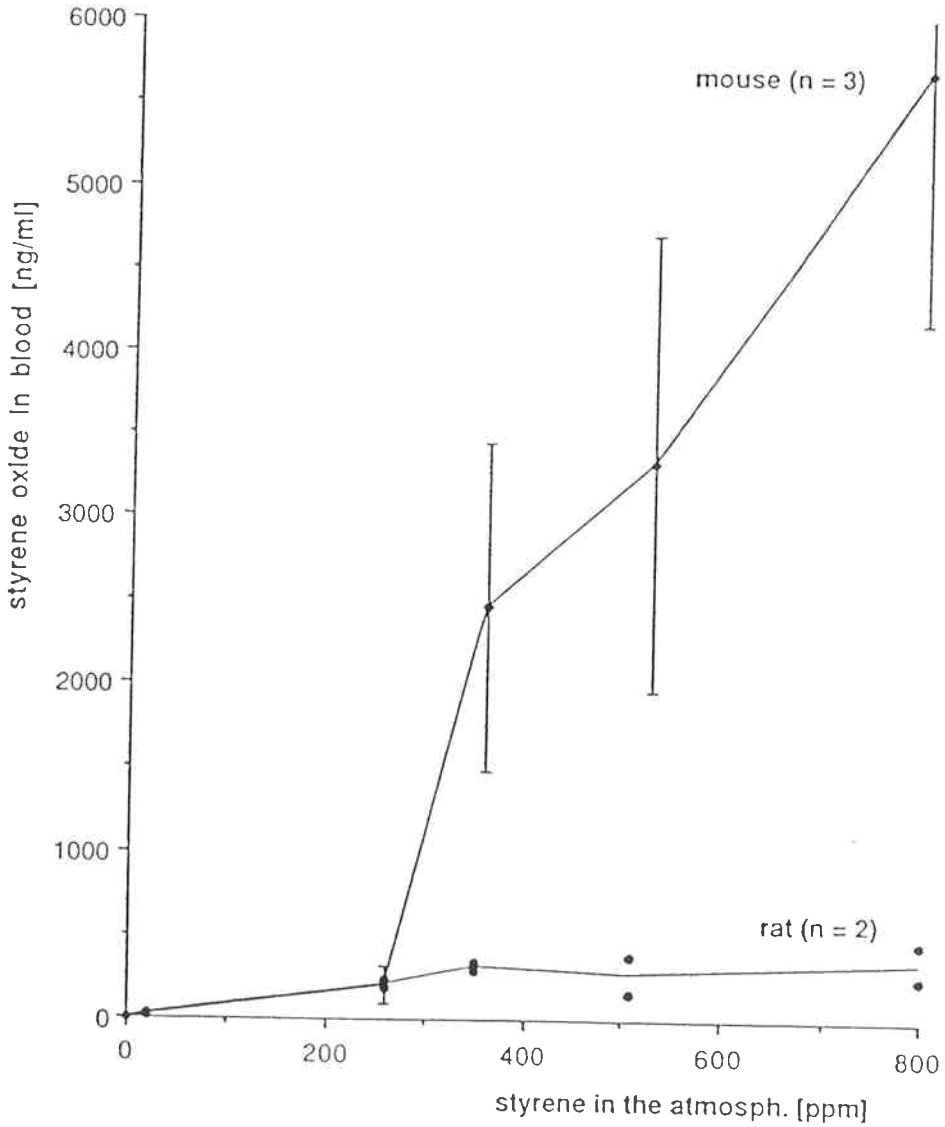
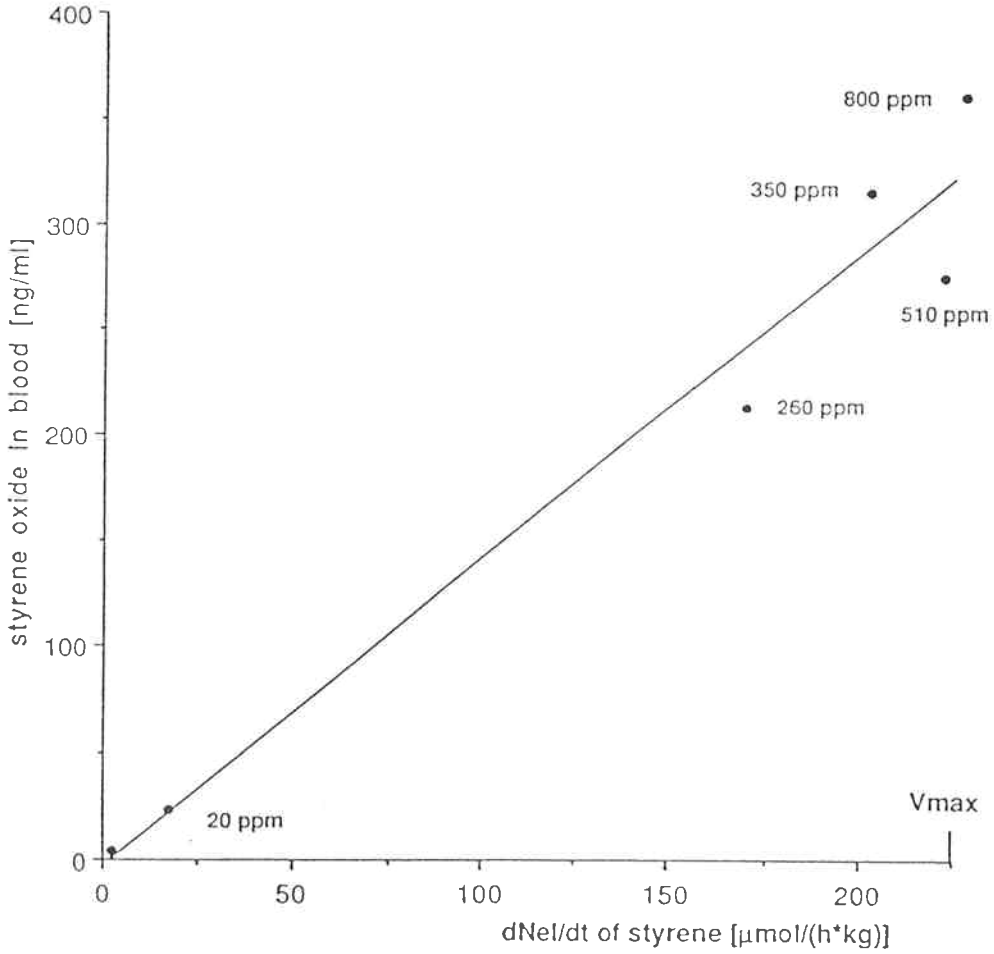


FIGURE 31

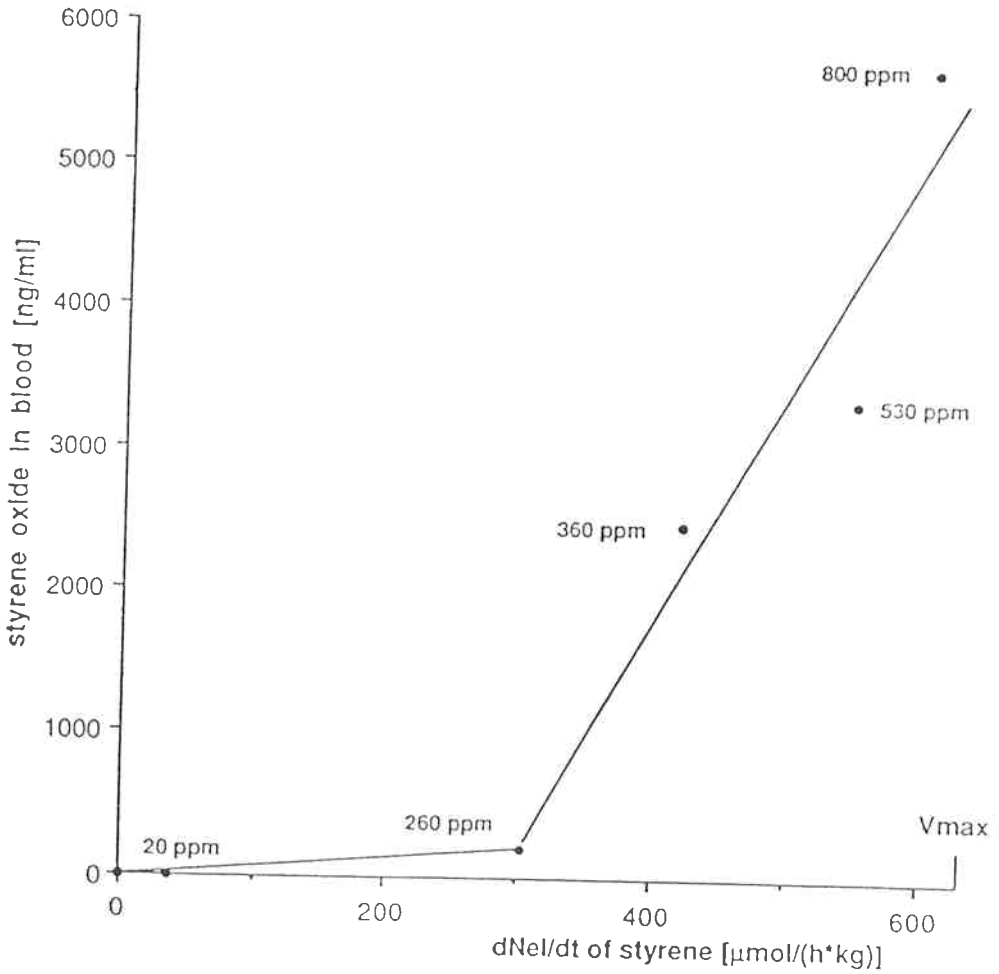
Correlation of styrene-7,8-oxide levels in blood at the end of steady state exposures to styrene with the calculated rates of styrene metabolism; rat



Symbols: means of measured values
Numbers: respective styrene concentrations
Vmax: maximal rate of metabolism of styrene

FIGURE 32

Correlation of styrene-7,8-oxide levels in blood at the end of steady state exposures to styrene with the calculated rates of styrene metabolism; mouse



Symbols: means of measured values
Numbers: respective styrene concentrations
Vmax: maximal rate of metabolism of styrene