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# Enzyme specific kinetics of 1,2-epoxybutene-3 in microsomes and cytosol from livers of mouse, rat, and man

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**Abstract.** Kinetics of the metabolism of 1,2-epoxybutene-3 (butadiene monoxide) were investigated in liver fractions of mouse, rat, and man. In these species similar enzyme characteristics were found. In microsomes, no NADPH-dependent metabolism of butadiene monoxide was detectable. Epoxide hydrolase activity was found only in microsomes. The $V_{max}$ [nmol butadiene monoxide/(mg protein × min)] was 19 in mouse, 17 in rat, and 14 in man and the apparent $K_m$ (mmol butadiene monoxide/l incubate) was 1.5 in mouse, 0.7 in rat, and 0.5 in man. Glutathione S-transferase activity was found in cytosol only, revealing first order kinetics in the measured range. The ratio $V_{max}/K_m$ [(nmol butadiene monoxide × l)/(mg protein × min × mmol of butadiene monoxide)] was 15 in mouse, 11 in rat, and 8 in man. The data obtained were used to extrapolate on the total rate of butadiene monoxide metabolism for each species in vivo: it was calculated to be 1.3 times higher in mice and 2.3 times lower in man compared to rats, when corrected for body weight.

**Key words:** 1,2-Epoxybutene-3 – Butadiene monoxide – Microsomes – Cytosol – Pharmacokinetics – Mouse – Rat – Man – Epoxide hydrolase – Glutathione S-transferase – Cytochrome P-450-dependent monooxygenase – In vivo extrapolation

## Introduction

1,2-Epoxybutene-3 (butadiene monoxide) was mutagenic in bacterial test systems (De Meester et al. 1978; Hemminki et al. 1980; Voogd et al. 1981) and carcinogenic in mice (Van Duuren et al. 1963). It is a reactive metabolite of 1,3-butadiene (butadiene) (Filser and Bolt 1984) which has also been shown to be carcinogenic. In long term inhalation studies the carcinogenic potency of butadiene was considerably higher in mice than in rats (Hazleton Labs. 1981; Huff et al. 1985). These findings might partly be explained by different target doses of butadiene monoxide resulting from differences in the rate of its formation and elimination.

A basis for risk estimation due to exposure to butadiene is the information on the kinetics of its biotransformation to butadiene monoxide and its further metabolism. For ethical reasons, it is not possible to expose humans to butadiene monoxide. Therefore its kinetic parameters have to be established by in vitro experiments.

In the livers of mice, rats, and man butadiene is oxidized to butadiene monoxide (Malvoisin et al. 1979; Schmidt and Loeser 1985; Bond et al. 1988). In liver fractions of rats this epoxide has been shown to be inactivated by microsomal epoxide hydrolase (Malvoisin and Roberfroid 1982) and by cytosolic glutathione S-transferase (Bolt et al. 1983). However, no data concerning the species-specific kinetics of these enzymes were available.

The aim of this study was to measure and to compare the kinetics of the enzymes metabolizing butadiene monoxide in microsomes and cytosol isolated from livers of mouse, rat, and man, and to extrapolate these results to conditions in vivo.

## Materials and methods

### *Animals and chemicals*

Livers were prepared from male NMRI mice (37–40 g body weight) and male Sprague-Dawley rats (350–450 g body weight) all inbred strains from GSF, Neuherberg (FRG). A specimen (130 g) of one healthy human liver was obtained from a male donor during an organ transplantation. Racemic 1,2-epoxybutene-3, 98% pure, was purchased from Aldrich, Steinheim (FRG). All other chemicals or biochemicals were obtained from Merck, Darmstadt (FRG), or from Boehringer, Mannheim (FRG), except glutathione (reduced form, GSH) and D, L-isocitric acid trisodium salt which were from Sigma, Deisenhofen (FRG), and Aldrich, Steinheim (FRG), respectively, in the purest analytical grade available.

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### *Preparation of liver fractions and incubation conditions*

Liver microsomes and liver cytosol were prepared according to DePierre and Morgenstern (1983). The microsomes obtained were washed at 0° C with a KCl solution of 0.15 mol/l. Subsequently, they were resuspended in iced buffer containing 0.05 mol/l tris(hydroxymethyl)aminomethane and 0.25 mol/l sucrose, pH = 7.4. Both fractions were stored at –80° C.

Incubations of up to 2 h were done at 37° C in 42-ml vessels containing 1 ml incubation medium which normally consisted of: liver microsomes or liver cytosol 3 mg protein/ml, K-phosphate buffer 150 mmol/l (pH 7.4), $MgCl_2$ 0.5 mmol/l, glutathione 15 mmol/l (GSH, pH 7.4), NADP 0.1 mmol/l, D,L-sodium-isocitrate 0.8 mmol/l, isocitrate dehydrogenase (pig heart) 50 µg/ml. Another series of experiments was carried out with microsomes as described, but the NADPH-regenerating system was added at ten-fold higher concentrations: $MgCl_2$ 5 mmol/l, NADP 1 mmol/l, D,L-Na-isocitrate 8 mmol/l, isocitrate dehydrogenase, unchanged, 50 µg/ml. Control experiments were carried out without the NADPH regenerating system, others with boiled liver fractions by omitting or adding glutathione. To several control samples of both fractions 2-chloroacetophenone 5 mmol/l was added instead of glutathione for depleting residual glutathione (Summer 1988, personalcommunication). The aim of these control experiments was to determine the extent of metabolism due to NADPH-dependent monooxygenase, to nonenzymic reaction of glutathione with butadiene monoxide, and to glutathione S-transferase, respectively.

Butadiene monoxide was administered to the gas phase of the vessels at initial atmospheric concentrations of 30, 300, and 3000 ppm by means of a modified system (Fig. 1) according to Kappus et al. (1976). Suitable concentrations of vaporized butadiene monoxide were adjusted in the storage chamber (A). The distributor (B) and the vessels (C) were evacuated to 2.7 kPa by a membrane vacuum pump. Meanwhile the vessels (C) were cooled by ice water. After opening the valve between A and B, identical concentrations of butadiene monoxide were obtained in all vessels. Then, by briefly opening the appropriate valve of A, atmospheric pressure was achieved. The vessels were disconnected after closing their valves and incubated in a shaking water bath. Subsequently one probe of 5 ml was withdrawn from the gas phase of each vessel.

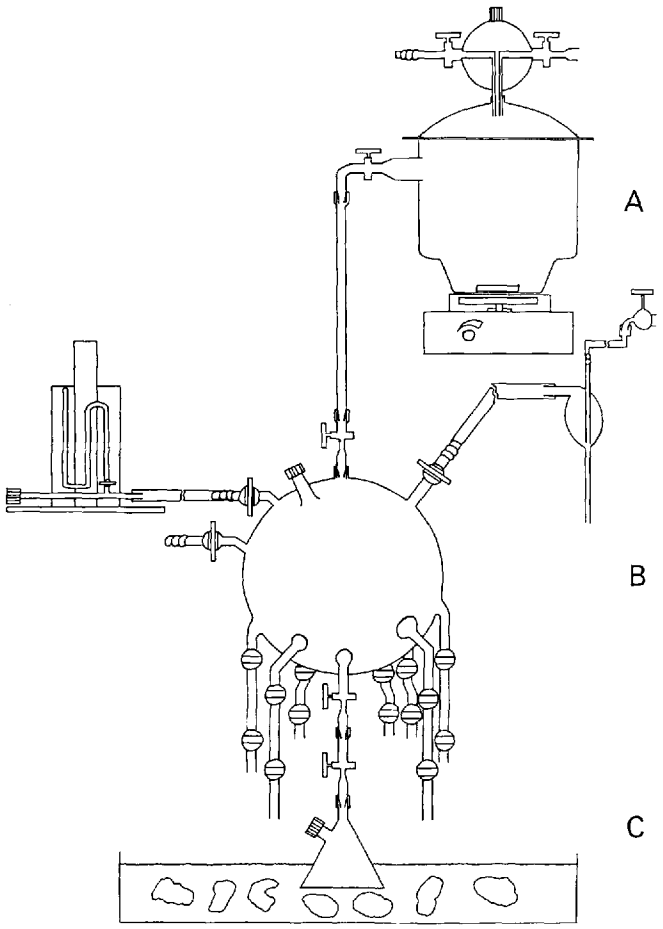


**Fig. 1.** Gas-dosing system for achieving equal gas concentrations in incubation vessels. *A*, storage chamber (20 l) with magnetic stirrer; *B*, distributor (4 l) with water pump and mercury manometer; *C*, incubation vessels (42 ml each) in iced water

### *Gas chromatography*

For analysis of the butadiene monoxide concentration the probe was injected via a 1 ml sample loop into a Packard 437 A gas chromatograph (United Technologies Packard, Frankfurt, FRG) equipped with a flame ionization detector. Separation was done at 85° C on a 1/8″ stainless steel column, length 0.6 m, filled with Tenax GC (60–80 mesh). Gas flows were 42 ml/min nitrogen as carrier gas, 216 ml/min synthetic air and 60 ml/min hydrogen. Detector temperature was kept at 225° C. Signals were integrated with a Shimadzu C-R6A integrator (Shimadzu, Duisburg, FRG). The precision of the method was evaluated from three independent experiments. The coefficients of variance were less than 8% of the means.

### *Kinetic analysis*

Metabolism of butadiene monoxide was analyzed by a pharmacokinetic two-compartment model adapted for metabolism according to Michaelis-Menten kinetics (Kessler et al. 1989) (Fig. 2). Since a nonenzymic reaction of glutathione with butadiene monoxide was observed, the first order rate constant ($k_{ne}$) was introduced into the 2-compartment model (Fig. 2), describing the nonenzymic elimination. To determine $k_{ne}$, experiments with glutathione and butadiene monoxide were carried out using microsomes and cytosol, inactivated by heating at 95° C for 15 min. Concentrations of butadiene monoxide in the liquid phase were calculated from the measured atmospheric concentrations using the obtained kinetic data.

In order to extrapolate rates of metabolism from findings in vitro to conditions in vivo, we made the following assumptions and calculations:

The body weight (body wt) of a mouse was standardized to 24 g, of a rat to 250 g, and of a man to 70 000 g. The liver weight (w) was set to 1 g in mouse, 10 g in rat, and 1500 g in man (Boxenbaum 1980). Protein content in the liver was assumed to be similar in all three species. The microsomal protein content was set to be 30 mg/g liver wet weight and the cytosolic one was assumed to be 95 mg/g wet weight (Alberts et al. 1983). Only the epoxide hydrolase- and the glutathione S-transferase-mediated metabolic pathways in the liver were taken into account for the extrapolation from in vitro to in vivo conditions.

We calculated the epoxide hydrolase-dependent rate of metabolism per kg body weight in vivo from the data obtained in vitro by the following equations:

$$V_{max}' = (V_{max} \times w \times n \times m \times 60)/1000 \quad (1)$$

$V_{max}'$: maximal rate of metabolism in vivo [µmol butadiene monoxide/ (h × kg body wt)]
$V_{max}$: maximal rate of metabolism in in vitro incubations [nmol butadiene monoxide / (min × mg protein)] (Fig. 2)
w: liver weight (g)
n: number of animals equivalent to 1 kg body wt (mouse: 42, rat: 4, man: 1/70)
m: microsomal protein concentration (30 mg/g)
60: factor for converting minute to 1 h
1000: divisor for converting nmol to µmol

Subsequently, we supposed an inner butadiene monoxide burden of the liver of 0.5 mmol/l. Since below this concentration, the rates of metabolism were directly proportional to the butadiene monoxide concentration in the incubates of the three species (see Results and discussion), we calculated the rate of epoxide hydrolase dependent metabo-

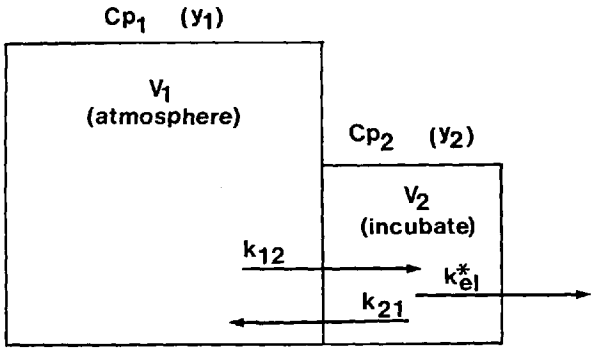


**Fig. 2.** Kinetic two-compartment model and differential equations for the exposure system.
Differential equations related to the kinetic model:
Atmosphere: $V_1 \times (dy_1/dt) = -k_{12} \times V_1 \times y_1 + k_{21} \times V_2 \times y_2$
Incubate: $V_2 \times (dy_2/dt) = k_{12} \times V_1 \times y_1 - (k^*_{el} + k_{21}) \times V_2 \times y_2$
$Cp_1$: compartment 1; $Cp_2$: compartment 2
$y_1$: concentration in the atmosphere (µmol/ml)
$y_2$: concentration in the incubate (µmol/ml)
$V_1$: volume of the gas phase (ml)
$V_2$: volume of the incubate (ml)
$k_{12}$: microconstant of uptake (1/h)
$k_{21}$: microconstant of discharge (1/h)
$k^*_{el}$: microconstant of metabolic elimination (concentration dependent) (1/h); in case of Michaelis-Menten kinetics $k^*_{el}$ is given by:
$k^*_{el} = V^*_{max}/[V_2 \times (K_m + y_2)] + k_{ne}$
$V^*_{max}$: maximal rate of metabolism in the incubate containing 3 mg protein (µmol/h); conversion of $V^*_{max}$ to $V_{max}$ [nmol/(min × mg protein)] by:
$V_{max} = V^*_{max} \times 1000/(60 \times 3)$
$K_m$: apparent Michaelis-Menten constant for a saturable pathway (concentration in $Cp_2$ at $V_{max}/2$) (µmol/ml = mmol/l)
$k_{ne}$: microconstant for nonenzymic elimination of butadiene monoxide from the incubate containing 15 mmol/l glutathione (1/h)

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lism at 0.5 mmol butadiene monoxide/l according to Michaelis-Menten kinetics:

$$v(0.5)EH = [V_{max}' \times y(0.5)]/[K_m + y(0.5)] \quad (2)$$

v(0.5)EH: rate of metabolism in vivo of epoxide hydrolase at 0.5 mmol butadiene monoxide/l liver tissue [µmol butadiene monoxide/(h × kg body wt)]
$K_m$: apparent Michaelis-Menten constant in vitro (mmol butadiene monoxide/l incubate)
y(0.5): butadiene monoxide concentration in the liver (mmol/l)

The glutathione S-transferase depending rate of metabolism per kg of body weight in vivo was calculated by the following equation:

$$v(0.5)GST = [v(y(0.5)) \times w \times n \times c \times 60]/1000 \quad (3)$$

v(0.5)GST: rate of metabolism in vivo of glutathione S-transferase at supposed 0.5 mmol butadiene monoxide/l liver tissue [µmol butadiene monoxide/(h × kg body wt)]
v(y(0.5)): rate of metabolism in vitro of glutathione S-transferase corresponding to 0.5 mmol butadiene monoxide/l incubate [nmol butadiene monoxide/(min × mg protein)]
w, n, 60, 1000: see above
c: cytosolic protein concentration (95 mg/g)

The rate constant for the nonenzymic reaction of butadiene monoxide with glutathione (15 mmol/l incubate) is given by $k_{ne}$ (Fig. 2). For calculation of the species specific rate constants $k_{neM}$, $k_{neR}$, and $k_{neH}$ for mouse, rat, and human, respectively, the glutathione concentrations in the livers of 9 mmol/l mouse (Jaeschke and Wendel 1985), 11 mmol/l rat (Farooqui and Ahmed 1984), and 3 mmol/l human (Summer and Eisenburg 1985) were used. The following constants have been obtained:

$$k_{neM} = k_{ne} \times (9/15)\ (1/h) \quad (4a)$$
$$k_{neR} = k_{ne} \times (11/15)\ (1/h) \quad (4b)$$
$$k_{neH} = k_{ne} \times (3/15)\ (1/h) \quad (4c)$$

The rate of nonenzymic reaction per kg body weight of the different species was calculated by the following equation:

$$v(0.5)ne = k_{neM/R/H} \times y(0.5) \times w \times n[5]$$

v(0.5)ne: rate of nonenzymic reaction in vivo at a supposed inner burden of 0.5 mmol butadiene monoxide/l liver tissue [µmol butadiene monoxide/(h × kg body wt)]
$k_{neM/R/H}$: $k_{neM}$ or $k_{neR}$ or $k_{neH}$
y(0.5), w, n: see above

The total rate of metabolism of butadiene monoxide in mouse, rat, and man in vivo was estimated by the following equation:

$$v(0.5)tot = v(0.5)EH + v(0.5)GST \quad (6)$$

v(0.5)tot: total rate of metabolism in vivo at a supposed inner burden of 0.5 mmol butadiene monoxide/l liver tissue [µmol butadiene monoxide/(h × kg body wt)].

## Results and discussion

### *Liver microsomes*

The data obtained from the standard incubates of microsomes from livers of mice, rats, and man and the best fits obtained by means of the kinetic model are shown in Fig. 3 *A–C*, respectively. According to the procedure, these concentration-time courses could represent the sum of the activities of cytochrome P-450 dependent monooxygenase, epoxide hydrolase, glutathione S-transferase and of a nonenzymic reaction of glutathione with butadiene monoxide. In order to distinguish between these pathways, we carried out control experiments.

Some incubations were done without the NADPH-regenerating system or with concentrations of $MgCl_2$, isocitrate, and NADP 10 times higher than in the standard procedure. Absence of the NADPH regenerating system did not reduce the elimination rate of butadiene monoxide, as shown with mice liver microsomes in Fig. 3*D*. At the high concentration of the NADPH-regenerating system no further increase was observed (data not shown).

In incubates with boiled microsomes of the three species we observed a nonenzymic reaction of glutathione with butadiene monoxide. This is shown in microsomes from mice livers in Fig. 3*D*. For this reaction a $k_{ne}$ value of 0.9 (1/h) ± 0.1 ($\bar{x} \pm$ SD; $n = 7$) was obtained for each of the three species, giving a half-life of 0.8 (h) ± 0.1 ($\bar{x} \pm$ SD) in the incubates.

A nonenzymic reaction was also reported by other groups (Malvoisin and Roberfroid 1982; Bolt et al. 1983).

After adding glutathione to incubates with microsomes of the three species, an increase of the rate of butadiene monoxide elimination from the atmosphere was observed. However, it did not exceed the rate of nonenzymic reaction, as shown with mice liver microsomes in Fig. 3*D*.

In liver microsomes of the three species neither metabolism of butadiene monoxide by NADPH-dependent monooxygenase nor by glutathione S-transferase was observed.

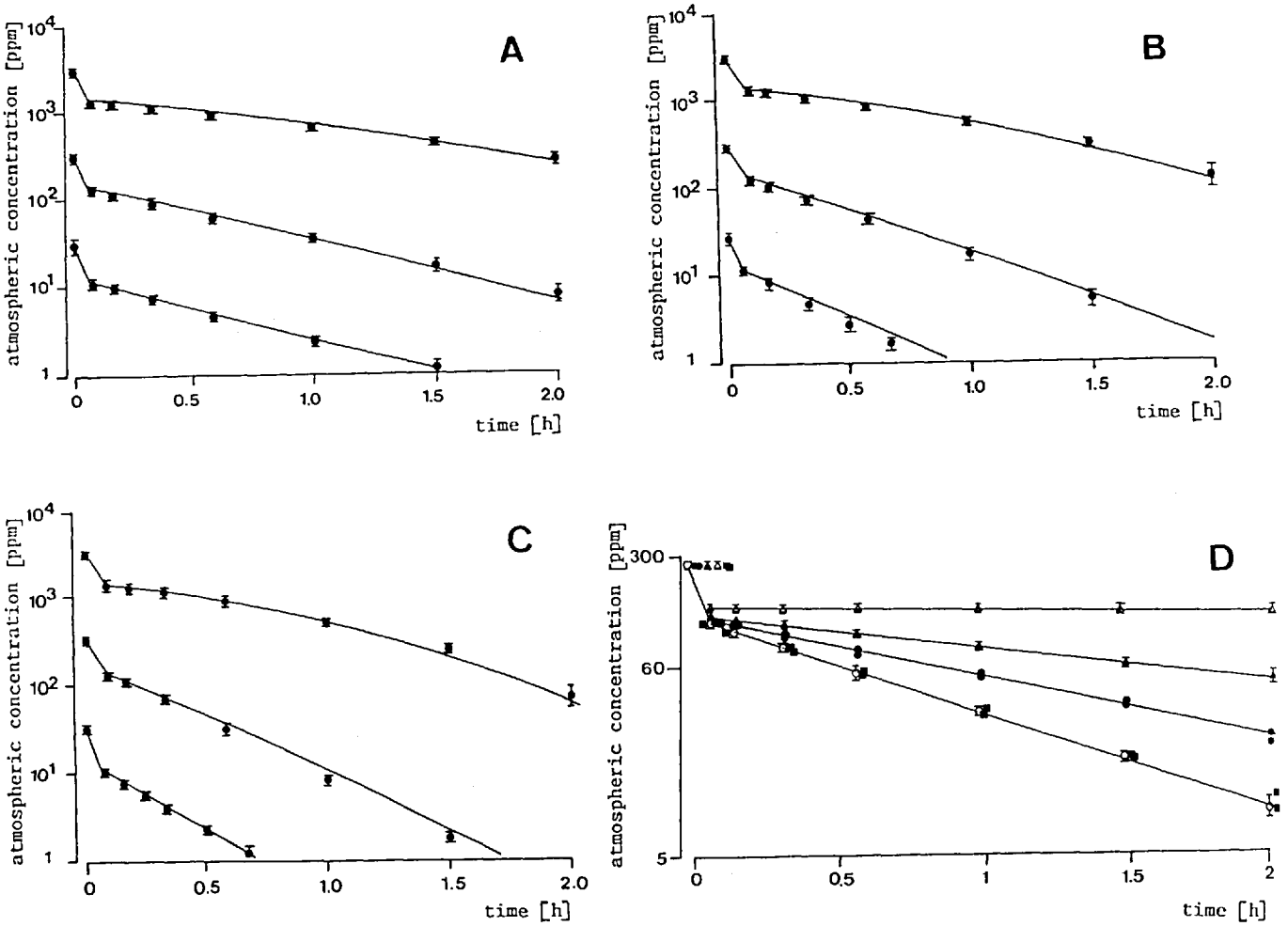


**Fig. 3 A–D.** Concentration-time courses of butadiene monoxide in the gas phase (41 ml) of closed incubation vessels containing incubates (1 ml) of liver microsomes. **A:** mouse (NMRI); **B:** rat (Sprague-Dawley); **C:** man; **D:** control incubates. Symbols: measured (means of 3 independent experiments +/– SD). Lines (in **A, B, C**): calculated using the two-compartment model; **D** fitted by eye. Incubations were carried out at 37° C. Standard incubates for **A, B,** and **C** contained: microsomes 3 mg protein/ml incubate, K-phosphate buffer 150 mmol/l (pH 7.4), $MgCl_2$ 0.5 mmol/l, glutathione 15 mmol/l (GSH, pH 7.4), NADP 0.1 mmol/l, D,L-sodium-isocitrate 0.8 mmol/l, isocitrate dehydrogenase 50 µg/ml. Incubates for **D** contained: (△) boiled (15 min) microsomes from mice livers in standard incubate without glutathione; (▲) boiled (15 min) microsomes from mice livers in standard incubates; (●) intact microsomes from mice livers in standard incubate without glutathione (double experiment); (■) intact microsomes from mice livers in standard incubate without NADPH-regenerating system (double experiment); (○) intact microsomes from mice livers in standard incubate

From these experiments and considering the detection limits of our method, it follows that the share of NADPH and glutathione dependent metabolic pathways could not exceed 9% of the microsomal butadiene monoxide metabolism. Therefore we conclude that in microsomes of the three species only epoxide hydrolase-dependent metabolism of butadiene monoxide was determined. This metabolism could be described by kinetics according to Michaelis-Menten (Fig. 5*A*, Table 1).

In the concentration range examined metabolism in liver microsomes of mice was strikingly lower than that of rats and man. The apparent $K_m$ in mice (1.5 mmol/l) was twice as high as in rats (0.7 mmol/l) and three times higher than in man (0.5 mmol/l). In all three species, $V_{max}$ were similar [mice 19, rats 17, man 14 nmol/(min × mg)].

Malvoisin and Roberfroid (1982) also exposed liver microsomes from rats to butadiene monoxide and derived a similar $V_{max}$ value [11 nmol/(min × mg protein)]; however, their corresponding $K_m$ value was much higher (11 mmol/l). Their incubations were made at a pH value of 9.0, whereas we incubated at the physiological pH of 7.4. Furthermore, Malvoisin and Roberfroid (1982) determined the kinetic parameters of the epoxide hydrolase by measuring the product 3-butene-1,2-diol indirectly.

In mice, the kinetic parameters of epoxide hydrolase-mediated metabolism are highly substrate specific. Depending on the substrates, liver microsomes showed $V_{max}$ values between 0.4 and 10 nmol/(min × mg protein) and $K_m$ values ranging from 0.065 to 0.24 mmol/l (Cantoni et al. 1978; Oesch 1979; Glatt et al. 1984; Lorenz et al. 1984; Del Monte et al. 1985; Longo et al. 1985; Magdalou and Hammock 1987). Therefore, butadiene monoxide had the lowest affinity of these substrates for which have been reported. The relative enzyme capacity is the highest.

In rats, with other substrates, $V_{max}$ values between 0.2 and 12 nmol/(min × mg protein) have been reported. The $K_m$ values ranged from 0.006 to 12 mmol/l (Oesch et al. 1971;Ryan et al. 1976; Cantoni et al. 1978; Friedberg et al.

**Table 1.** Pharmacokinetic parameters of butadiene monoxide in microsomes and cytosol of mouse, rat, and man

| Species | Microsomes (epoxide hydrolase activity) | | Cytosol (glutathione S-transferase activity) |
|---|---|---|---|
| | $V_{max}$ | $K_m$ | $V_{max} / K_m$ |
| | $\left[\frac{nmol}{min \times mg}\right]$ | $\left[\frac{mmol}{l}\right]$ | $\left[\frac{nmol \times l}{min \times mg \times mmol}\right]$ |
| Mouse | 19 | 1.5 | 15 |
| Rat | 17 | 0.7 | 11 |
| Man | 14 | 0.5 | 8 |

$V_{max}/K_m$ was obtained from $k_{el}^*$ (Fig. 2) after subtraction of $k_{ne}$, by multiplying by $[1000/(3 \times 60)]$ for correction of dimension

1979; Oesch 1979; Malvoisin and Roberfroid 1982; Glatt et al. 1984; Lorenz et al. 1984; Longo et al. 1985; Denlinger and Vesell 1989). Considering these data, epoxide hydrolase seems to have a high capacity for butadiene monoxide which is a substrate with medium affinity.

In man for liver microsomes different groups reported $V_{max}$ values between 5 and 6800 nmol/(min × mg protein) depending on the substrate (Oesch et al. 1974; Glatt et al. 1980; Lorenz et al. 1984; Mertes et al. 1985; Schmidt and Loeser 1985). The relative epoxide hydrolase capacity for butadiene monoxide in our system was therefore low. Oesch et al. (1974) reported a $K_m$ value of 0.4 mmol/l for purified epoxide hydrolase from liver homogenate using styrene oxide as substrate. The $K_m$ value found by us for butadiene monoxide as substrate was similar.

The NADPH-dependent pathway would lead to 1,2:3,4-diepoxybutane, which is also mutagenic and carcinogenic (Van Duuren et al. 1963; De Meester 1988). Up to now, it is not clear whether diepoxybutane is formed as

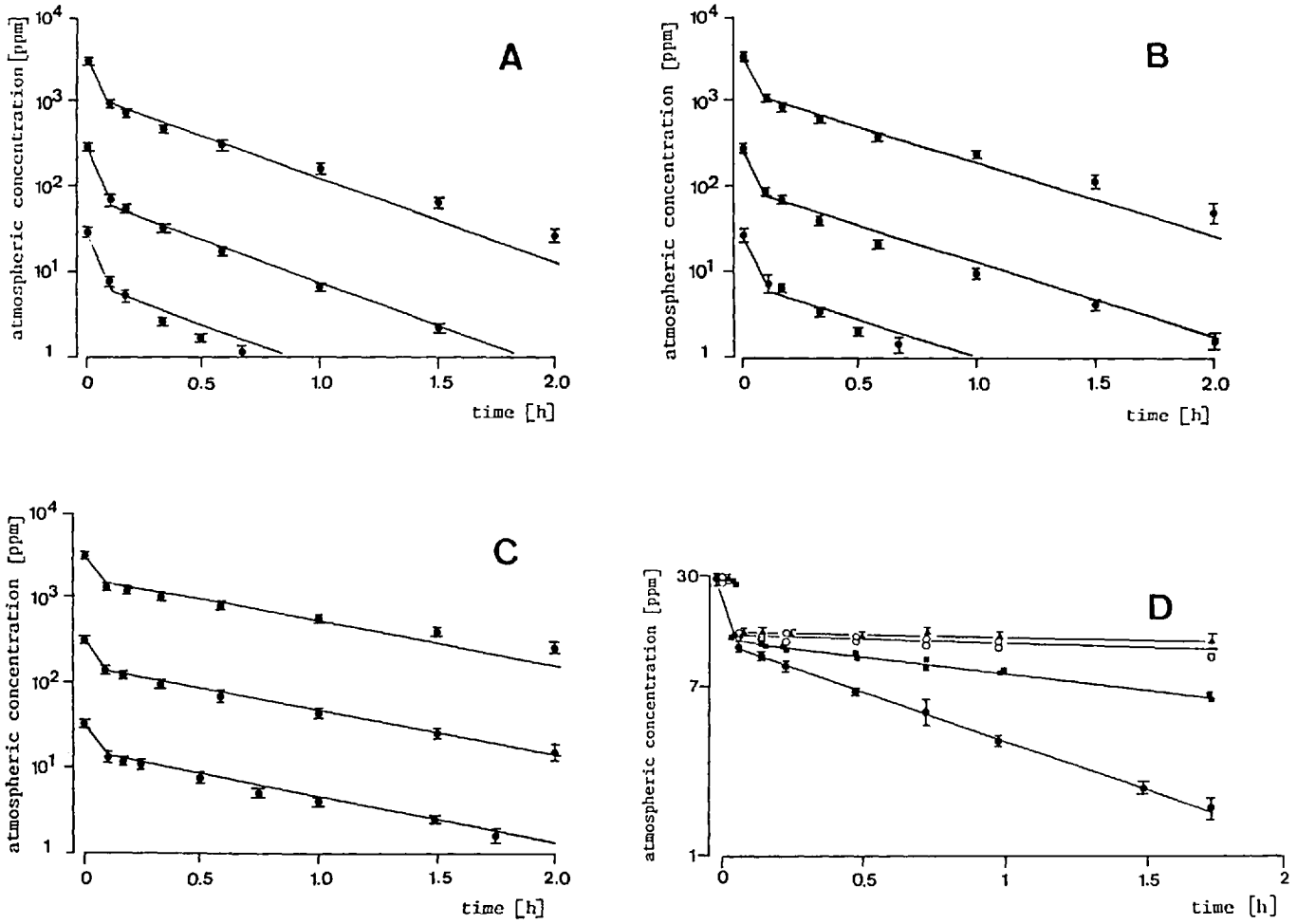


**Fig. 4 A–D.** Concentration-time courses of butadiene monoxide in the gas phase (41 ml) of closed incubation vessels containing incubates (1 ml) of liver cytosol. **A:** mouse (NMRI); **B:** rat (Sprague-Dawley); **C:** man; **D:** control incubates. Symbols: measured (means of 3 independent experiments +/– SD). Lines (in **A, B, C**): calculated using the two-compartment model; **D** fitted by eye. Incubations were carried out at 37° C. Standard incubates for **A, B,** and **C** contained: cytosol 3 mg protein/ml incubate, K-phosphate buffer 150 mmol/l (pH 7.4), $MgCl_2$ 0.5 mmol/l, glutathione 15 mmol/l (GSH, pH 7.4), NADP 0.1 mmol/l, D, L-sodium-isocitrate 0.8 mmol/l, isocitrate dehydrogenase 50 µg/ml. Incubates for D contained: (▲) boiled (15 min) cytosol from human liver in standard incubate without glutathione; (○) intact cytosol from human liver in standard incubate without glutathione, depletion of residual glutathione with 2-chloroacetophenone (5 mmol/l; double experiment); (■) boiled (15 min) cytosol from human liver in standard incubate (double experiment); (●) intact cytosol from human liver in standard incubate

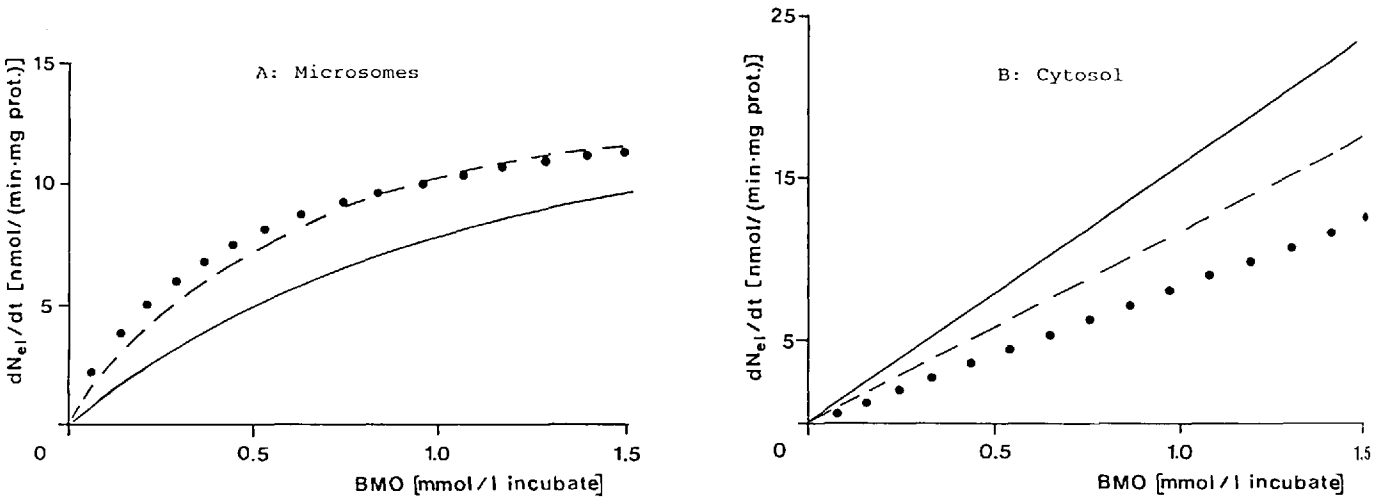


**Fig. 5.** Calculated curves giving the dependence of metabolic rates on the concentrations of butadiene monoxide in liver microsomes (A) or cytosol (B) of mice, rats, and one man, related to the liquid phase. ———: mice; – – – –: rats; ·····: man

a metabolite of 1,3-butadiene. Malvoisin and Roberfroid (1982) reported the production of diepoxybutane by rat liver microsomes treated with butadiene monoxide, but Schmiedel (1982) and Wistuba (1986) could not confirm these findings. Recently Ristau et al. (1990) exposed rats and mice to 1,3-butadiene with the aim to determine in the purified DNA crosslinks caused by reaction with the possible metabolite diepoxybutane. However, no crosslinks were found either in livers of mice or of rats. Carpenter et al. (1944) exposed rabbits to atmospheric 1,3-butadiene concentrations of 0.67% and 20–25% and measured the urinary excretion of the diepoxybutane metabolite erythritol. These authors also did not see formation of diepoxybutane, since no significant difference in the excretion of erythritol between exposed and control animals was observed.

The activities of liver microsomal and cytosolic glutathione S-transferases were studied in mouse, rat and man with 1-chloro-2,4-dinitrobenzene as substrate (Morgenstern et al. 1982; De Pierre and Morgenstern 1983; Lorenz et al. 1984; Andersson et al. 1988; McLellan et al. 1989). Activity was found in both cell fractions. In microsomes of mice, it was almost equal to that in cytosol. In microsomes of rats and man, it was about 10 times and up to 100 times lower, respectively. In none of these species, microsomal glutathione S-transferase activity was detected by us with butadiene monoxide as substrate.

*Liver cytosol*

The measured data using standard incubates of cytosol of the three species and the best fits obtained by means of the kinetic model are shown in Fig. 4*A–C*. Since these concentration-time courses could represent the sum of different enzymic activities and of the nonenzymic reaction with glutathione, we carried out control experiments in order to distinguish between these activities.

In incubates with boiled cytosol a nonenzymic reaction was observed for each species and the same results were obtained as in microsomes, as shown with human liver cytosol in Fig. 4*D*. The metabolism was completely inhibited after depletion of glutathione by 2-chloroacetophenone (Fig. 4*D*). Therefore, in liver cytosol of the three species, enzyme mediated metabolism of butadiene monoxide was exclusively related to glutathione S-transferase (Fig. 4*D*); i.e. no epoxide hydrolase-dependent metabolism could be detected.

Since the rates of glutathione S-transferase-mediated metabolism were directly proportional to the butadiene monoxide concentrations in the incubates, the concentration ranges were far below $K_m$. Only the relations of $V_{max}/K_m$ could be calculated (Fig. 5*B*, Table 1). At any concentration, the rate of metabolism was highest in liver cytosol of mice, followed by that of rats and man (Fig. 4*A–C*). Similar findings have been obtained comparing species differences with 1-chloro-2,4-dinitrobenzene (Bolt et al. 1981; De Pierre and Morgenstern 1983; Lorenz et al. 1984) and methylene chloride (Reitz et al. 1988) as substrates for cytosolic liver glutathione S-transferase. The activities of cytosolic glutathione S-transferases found in livers of mouse, rat, and man, were highly substrate specific, ranging over 3 orders of magnitude (Ryan et al. 1976; Bolt et al. 1981; De Pierre and Morgenstern 1983; Oesch et al. 1983; Lorenz et al. 1984; Tischler and Allen 1985; Magdalou and Hammock 1987; Pacifici et al. 1987; Reitz et al. 1988).

Concerning the activity of cytosolic epoxide hydrolase, few data are available. Using styrene oxide as substrate in liver cytosol from rats, the specific activity was 10 times lower than in microsomes (Oesch et al. 1971)

*Extrapolation from findings in vitro to conditions in vivo*

The kinetic parameters presented in Table 1 were used to extrapolate to conditions in vivo as described above. The maximal rate of epoxide hydrolase-mediated metabolism of butadiene monoxide ($V_{max}'$) was obtained to be 1440 [μmol/(h × kg body wt)] in mice, 1220 [μmol/(h × kg body

Table 2. Rates of metabolism of butadiene monoxide calculated for conditions in vivo

| Species | v(0.5)EH* | v(0.5)GST** | v(0.5)ne*** | v(0.5)tot**** |
|---|---|---|---|---|
| | [µmol butadiene monoxid / h × kg body weight] | | | |
| Mouse | 400 | 1900 | 10 | 2300 |
| Rat | 500 | 1300 | 10 | 1800 |
| Man | 300 | 500 | 2 | 800 |

The rates are referred to 1 kg body weight and are calculated for butadiene monoxide burden in the liver of 0.5 mmol/l tissue

* Rate of epoxide hydrolase dependent metabolism

** Rate of glutathione S-transferase dependent metabolism

*** Rate of nonenzymic metabolism

**** Total rate of metabolism

wt)] in rats and 540 [µmol/(h × kg body wt)] in man. The lowest $K_m$ of 0.5 mmol/l liver tissue was obtained in man. Since below this value the concentration-metabolism curves can be treated as linear, we calculated the rate of butadiene monoxide metabolism in the livers of the three species at this concentration. For the extrapolation we assumed that butadiene monoxide, metabolized from butadiene, would be available in the liver equally for epoxide hydrolase and glutathione S-transferase. The results are given in Table 2.

In order to estimate the rate of the nonenzymic reaction of butadiene monoxide with glutathione in vivo, we extrapolated from the rate constant $k_{ne}$ in vitro to respective rate constants $k_{neM/R/H}$ in vivo. In rats, the metabolism of butadiene takes place mainly in the liver. Just one-third of the butadiene monoxide formed is systemically available (Filser and Bolt 1984). Furthermore, the concentration of glutathione is highest in liver compared to that in other organs (Farooqui and Ahmed 1984; Jaeschke and Wendel 1985). For these reasons only the liver was taken into account for the calculation of the rate of the nonenzymic reaction in vivo. The rate constants $k_{neM} = 0.5$ (1/h), $k_{neR} = 0.7$ (1/h) and $k_{neH} = 0.2$ (1/h) give rise to rates which are negligible compared to the respective enzymic rates (Table 2).

Based on body weight, the rate of estimated butadiene monoxide detoxification is 1.3 times higher in mice and 2.3 times lower in man compared to rats. Species scaling by means of a common allometrical method takes the body surfaces of mice, rats, and man into account. It leads to the same ranking but gives higher differences between the species: considering the body surface to be proportional to two-thirds of the power of the body volume, the rate of butadiene monoxide metabolism is expected to be about 5 times lower in one mouse of 24 g and 40 times higher in one man of 70 kg than in one rat of 250 g. Standardizing these data on body weight, mice are expected to metabolize 2 times faster and man 7 times slower than rats. In studies in vivo with mice and rats butadiene monoxide metabolism was indeed 2 times faster in mice compared to rats at exposure concentrations in which the rate of metabolism was proportional to the concentration (Kreiling et al. 1987). The two procedures give remarkably similar estimates in spite of the large difference in methodology. Therefore, we assume that butadiene monoxide metabolism in man would be between 2 and 7 times less effective than in rats.

In vivo, the body burden of butadiene monoxide is determined by both the rate of its formation from the metabolic precursor butadiene and of its elimination. In man, pharmacokinetics of butadiene have not yet been established. In mice, butadiene was metabolized about 2 times faster than in rats when it was normalized to body weight (Kreiling et al. 1986). Since in both species the relative formation of butadiene monoxide from butadiene and its elimination were similar, one should expect the concentration of butadiene monoxide to be within the same range in both species. Bond et al. (1986) exposed mice and rats to different concentrations of butadiene and determined the body burden with butadiene monoxide. Its concentration in the blood was 2–5 times higher in mice than in rats after 6 h of exposure. Similar findings were obtained by Kreiling et al. (1987), who exposed mice and rats in closed chambers to butadiene concentrations high enough to cause maximal rates of metabolism to butadiene monoxide for both species. During the first 5 h of exposure the concentration of exhaled butadiene monoxide was 1.5–2 times higher in the chambers containing mice, but it increased sharply with further extending exposure. In contrast, in rats a plateau was reached which remained constant until the end of exposure. The difference could be explained by the findings that in mice the hepatic non-protein sulfhydryl content was depleted to 4% after continuous exposure over 15 h, whereas in Sprague-Dawley rats it was depleted only to 76% (Kreiling et al. 1987, 1988). According to our findings, a complete loss of the glutathione dependent metabolic pathway of butadiene monoxide in mice results in about 20% of the initial detoxification capacity if butadiene monoxide metabolism is then mediated by epoxide hydrolase only (Table 2). This leads to increased concentration of the genotoxic butadiene monoxide (Citti et al. 1984) in the bodies of mice exposed to butadiene. The higher carcinogenic potency of butadiene which has been observed in mice (Huff et al. 1985) compared to rats (Hazleton 1981) might be explained by higher butadiene monoxide levels. Moreover, higher butadiene monoxide burden leads to a higher probability of the formation of the carcinogenic 1,2:3,4-diepoxybutane.

Extrapolated from one human liver, the detoxification of butadiene monoxide was much slower per kg body weight than in mice or rats (see above). However, since the pharmacokinetics of butadiene have not yet been established in man, the body burden of its metabolite, butadiene monoxide, cannot be estimated. Therefore, a risk estimate for man based on the presented data is not yet possible.

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