

Inhalation Pharmacokinetics of 1,3-Butadiene and 1,2-Epoxybutene-3 in Rats and Mice

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Studies were conducted on inhalation pharmacokinetics of 1,3-butadiene and of its primary reactive metabolic intermediate 1,2-epoxybutene-3 in rats (Sprague-Dawley) and mice (B6C3F₁). Investigations of inhalation pharmacokinetics of 1,3-butadiene revealed saturation kinetics of 1,3-butadiene metabolism in both species. For rats and mice linear pharmacokinetics apply at exposure concentrations below 1000 ppm 1,3-butadiene; saturation of 1,3-butadiene metabolism is observed at atmospheric concentrations of about 2000 ppm. The estimated maximal metabolic elimination rates were 400 $\mu\text{mole/hr/kg}$ for mice and 200 $\mu\text{mole/hr/kg}$ for rats. This shows that 1,3-butadiene is metabolized by mice at about twice the rate of rats. Investigations of inhalation pharmacokinetics of 1,2-epoxybutene-3 revealed major differences in metabolism of this compound between both species. No indication of saturation kinetics of 1,2-epoxybutene-3 metabolism could be observed in rats up to exposure concentrations of 5000 ppm, whereas in mice the saturation of epoxybutene metabolism became apparent at atmospheric concentrations of about 500 ppm. The estimated maximal metabolic rate for 1,2-epoxybutene-3 was 350 $\mu\text{mole/hr/kg}$ in mice and > 2600 $\mu\text{mole/hr/kg}$ in rats. When the animals are exposed to high concentrations of 1,3-butadiene, 1,2-epoxybutene-3 is exhaled by rats and mice. For rats 1,2-epoxybutene-3 concentration in the gas phase of the system reaches a plateau at about 4 ppm. For mice, 1,2-epoxybutene-3 concentration increases with exposure time until, at about 10 ppm, signs of acute toxicity are observed. Under these conditions hepatic nonprotein sulfhydryl compounds are virtually depleted in mice but not in rats. After exposure of rats and mice to (1,4-¹⁴C)1,3-butadiene, covalent binding of (¹⁴C)butadiene-derived radioactivity could be detected. In both species, comparable amounts of radioactivity were associated with liver DNA. Covalent binding to nucleoproteins was twice as high in mice when compared to rats, and thus it paralleled the higher metabolic rate for 1,3-butadiene in this species. We conclude, that in addition to the higher metabolism of 1,3-butadiene in mice, limited detoxification and thus accumulation of its primary reactive intermediate 1,2-epoxybutene-3 must be a major determinant for the higher susceptibility of mice to 1,3-butadiene-induced carcinogenesis.

Introduction

Long-term inhalation studies with rats and mice have demonstrated remarkable species differences in the carcinogenic potency of 1,3-butadiene. An inhalation carcinogenicity study in mice (B6C3F₁) exposed to 625 ppm and 1225 ppm 1,3-butadiene for 60 weeks (1) showed a marked increase in the incidence of primary tumors including lymphomas, hemangiosarcomas of the heart, lung adenomas, and carcinomas. In male and female rats

(Sprague-Dawley) exposed to 2000 ppm and 8000 ppm 1,3-butadiene for 110 weeks (2) there was also an increased incidence of tumors, but the types of tumors (e.g., mammary, thyroid follicular cell adenomas, uterine) differed from those seen in mice. Furthermore, in contrast to the study in rats that did not show mortality secondary to neoplasia, the study in mice had to be terminated after 60 weeks because of fatal tumors that reduced survival (1).

In microsomal incubates 1,3-butadiene is metabolized in the presence of a NADPH-regenerating system by cytochrome P-450 to its epoxide 1,2-epoxybutene-3 (3-5). Further metabolic transformation of the epoxide intermediate by epoxide hydrolase and/or monooxygenase would lead to 3,4-epoxy-1,2-butanediol (via 3-butene-1,2-diol) and to diepoxybutane (4,6). 1,2-Epoxybutene-3 is conjugated with glutathione (4) and can be metabolized by glutathione-S-transferase (5).

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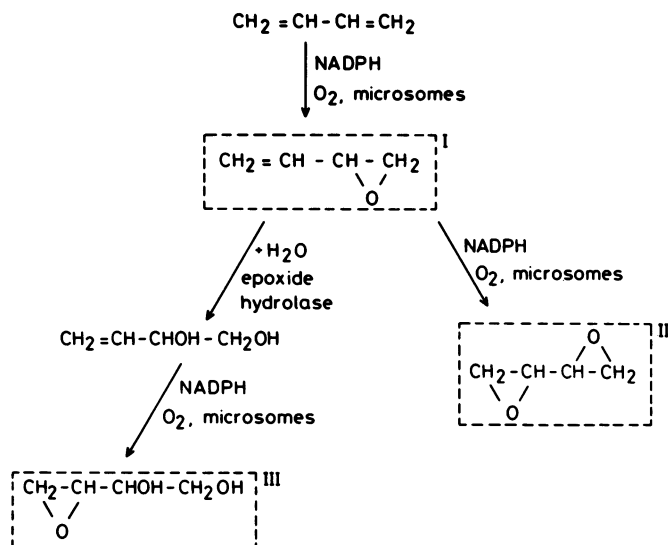


FIGURE 1. Metabolism of 1,3-butadiene according to Malvoisin et al. (6). The potential reactive intermediates, 1,2-epoxybutene-3 (I), diepoxybutane (II), and 3,4-epoxy-1,2-butane-diol (III) are framed.

Recently the formation of 1,2-epoxybutene-3 from 1,3-butadiene was demonstrated in organ homogenates of rats and mice (7). In addition, 1,2-epoxybutene-3 and diepoxybutane could be characterized, by vacuum-line cryogenic distillation, in the blood of rats and mice after exposure of the animals to ^{14}C -butadiene (8). Figure 1 shows the metabolism of 1,3-butadiene according to a proposal by Malvoisin and Roberfroid (6).

The purpose of our studies was to investigate whether the higher susceptibility of mice to 1,3-butadiene (when compared to rats) was due to quantitative differences in 1,3-butadiene metabolism between these two species. Therefore, comparative studies have been conducted in rats (Sprague-Dawley) and mice (B6C3F₁) on inhalation pharmacokinetics of 1,3-butadiene and of its reactive metabolic intermediate 1,2-epoxybutene-3. Furthermore, 1,3-butadiene-induced depletion of the hepatic nonprotein sulphhydryl (NPSH) content and the covalent binding of ^{14}C -butadiene to liver nuclear proteins and DNA were investigated in both species.

Methods

The methodological details of these investigations have already been published (9-12). Thus, only a brief summary is given here of the methods used.

Gas Uptake and Kinetic Studies

Male Sprague-Dawley rats (150-280 g) and male B6C3F₁ mice (25-30 g) were used for the experiments. Usually two rats or eight mice were placed in a closed 6.4-L desiccator jar, equipped with an O₂ supply and 135 g soda lime for CO₂ absorption (Fig. 2, top). The animals were exposed to initial concentrations of 1,3-butadiene and 1,2-epoxybutene-3 between about 10 ppm and 5000 ppm. Concentration changes of the gaseous

compounds were measured by gas chromatography after injecting either 1,3-butadiene or 1,2-epoxybutene-3 into the system or after administering the compounds IP to the animals. In some experiments diethylthiocarbamate, a metabolic inhibitor of cytochrome P-450, was administered IP at a single dose of 300 mg/kg body weight.

Kinetic parameters were determined from the concentration time-courses thus obtained, based on a two-compartment, open pharmacokinetic model that was developed by Filser and Bolt (13,14). This model implies a one-compartment description of the experimental animal. The gas phase in the desiccator with volume V_1 represented compartment one (Cp1), the animals with volume V_2 , compartment two (Cp2) (Fig. 2, bottom).

The full details of the analytical procedures and the pharmacokinetic analysis have been presented elsewhere (10,11,15,16).

Exhalation of 1,2-Epoxybutene-3

Six mice (B6C3F₁) or two rats (Sprague-Dawley) were placed in a 6.4-L desiccator system as described above. The animals were exposed to 1,3-butadiene at

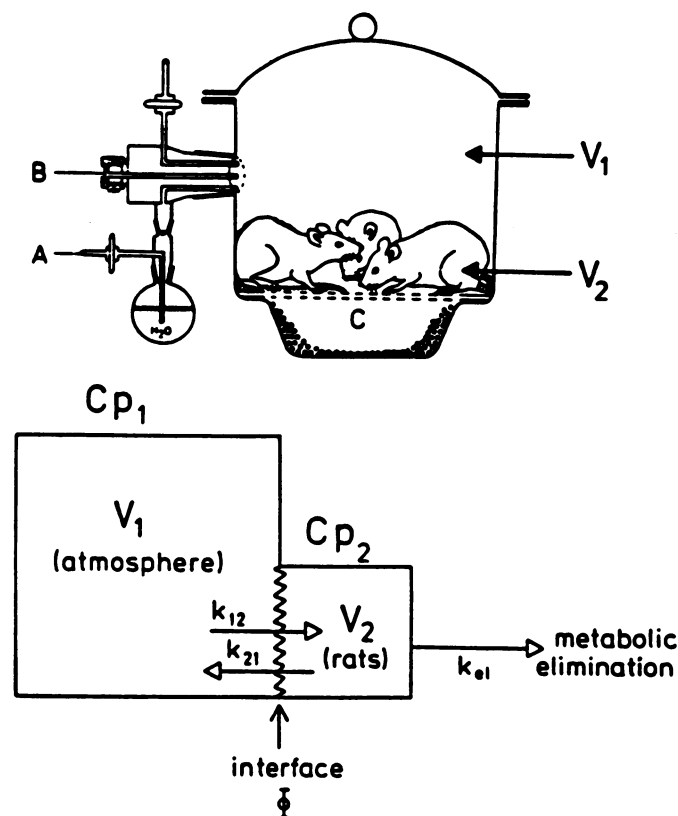


FIGURE 2. Top: Exposure system for the study of inhalation pharmacokinetics (13,14). (A) Oxygen inlet, (B) septum for injection of volatile compounds and for gas sampling, (C) soda lime. Bottom: Pharmacokinetic model with the compartments Cp1 (gas phase) and Cp2 (animals), the dividing theoretical interphase I and the rate constants of the partial process of invasion (k_{12}), exhalation (k_{21}), and first-order metabolic elimination (k_{e1}).

concentrations higher than 2000 ppm (2000–4000 ppm), which ensured maximal metabolism of the gas to 1,2-epoxybutene-3. Concentration changes of 1,3-butadiene and exhaled 1,2-epoxybutene-3 in the gas phase of the system were monitored by gas chromatography. The 1,3-butadiene concentration in the system was maintained above 2000 ppm for up to 17 hr (11).

Exposure of Animals to 1,3-Butadiene and Nonprotein Sulfhydryl Assay

Male B6C3F₁ mice (30–35 g), male Sprague-Dawley rats (180–200 g), and male Wistar rats (180–200 g) were used. For each individual experiment, six mice or three rats were placed in a 6.4-L all-glass exposure system and were exposed for up to 15 hr to 1,3-butadiene concentrations between 2000 to 3000 ppm (V_{\max} – conditions) to ensure maximal metabolism to 1,2-epoxybutene-3. Concentration changes were measured by gas chromatography. Control animals were also housed in closed all-glass chambers, but they were not exposed to 1,3-butadiene. After an exposure period of 7 hr or 15 hr, the animals were removed from the system and hepatic nonprotein sulfhydryl (NPSH) contents were determined according to Ellmann (17). The depletion of hepatic NPSH content was then expressed in % \pm SD of the respective control values. The full experimental details of this study have been presented (12).

Exposure of Animals to (1,4-¹⁴C)1,3-Butadiene and Isolation of Nuclear Proteins and DNA

Male B6C3F₁ mice (30–35 g) and male Wistar rats (200–220 g) were exposed in a 6.4-L all-glass desiccator to (1,4-¹⁴C) 1,3-butadiene (specific radioactivity 11.2 mCi/mmol). For each individual experiment 24 mice or 4 rats were used. Concentration changes of (¹⁴C)1,3-butadiene in the gas phase of the system were monitored by gas chromatography. After an exposure period of 6.6 hr (rats) or 4 hr (mice), more than 98% of the radioactivity was taken up by the animals. Total radioactivity uptake was 2.7 mCi per kilogram body weight for both species. Liver nucleoproteins and DNA were isolated from the purified nuclei (18) by hydroxylapatite chromatography (19). Radioactivity of the samples was determined by liquid scintillation counting and was related to protein or DNA content. The full experimental details of this study have been presented elsewhere (9).

Results and Discussion

Inhalation Pharmacokinetics of 1,3-Butadiene

Starting from different initial concentrations between 100 and 5000 ppm, the time-dependent decline of 1,3-butadiene in the exposure system, occupied by rats or mice, was investigated (10,15). The decline curves observed in these experiments for rats or mice (the time

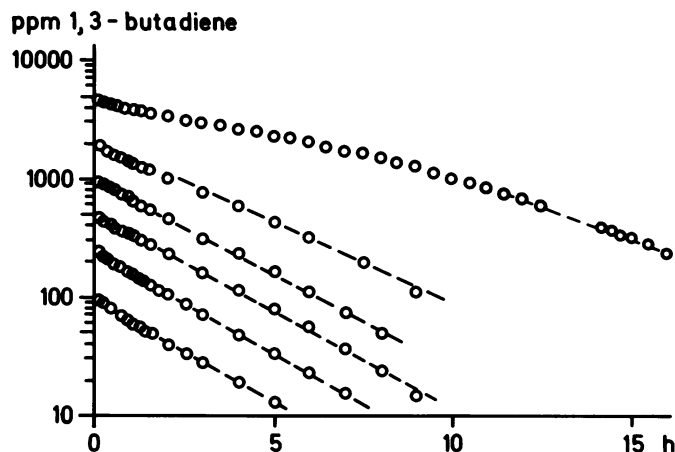


FIGURE 3. Time course of 1,3-butadiene concentrations in the gas phase of a closed 6.4-L desiccator jar chamber occupied by eight B6C3F₁ mice. Individual experiments with different initial 1,3-butadiene concentrations between 100 and 5000 ppm.

course of 1,3-butadiene concentrations are shown for mice in Figure 3) become flatter at higher exposure concentrations, indicating saturable metabolism of 1,3-butadiene in both species. Below concentrations of about 1000 ppm, the elimination of 1,3-butadiene by rats or mice can be described by a first-order process. At higher atmospheric concentrations saturation kinetics become apparent. Saturation of 1,3-butadiene metabolism is observed in rats and mice at atmospheric concentrations of about 2000 ppm. The pharmacokinetic parameters for distribution and metabolism of 1,3-butadiene were determined from the concentration-decline curves obtained (10,15) (Table 1). They show, in principle, that 1,3-butadiene is metabolized by mice at about twice the rate of rats. In the lower concentration range, where first-order metabolism applies, metabolic clearance per kg body weight was 7300 mL/hr for mice and 4500 mL/hr for rats. The estimated maximal metabolic elimination rates were 400 μ mole/hr/kg for mice and 220 μ mole/hr/kg for rats.

Figure 4 shows the metabolic elimination rates of 1,3-butadiene for rats and mice, calculated for conditions of exposure in an open ($V_1 \rightarrow \infty$) exposure system (13).

Table 1. Pharmacokinetic parameters for distribution and metabolism of 1,3-butadiene in mice and rats related to 1 kg body weight.^a

Parameter	Mouse	Rat	Dimension
$k_{12}V_1$	10,280	5,750	mL/hr
k_{21}	3.2	2.5	hr ⁻¹
K_{eqa}	2.7	2.3	—
K_{stb}	1.0	0.5	—
k_{el}	7.6	8.8	hr ⁻¹
$Cl_{tot}^{b,c}$	7,300	4,500	mL/hr
V_{max}	400	220	μ mole/hr/kg

^aFor definition of parameters see text and Filser and Bolt (13).

^bCalculated for $V_1 \rightarrow \infty$.

^cValid for linear range of metabolism (up to 1000 ppm for both species).

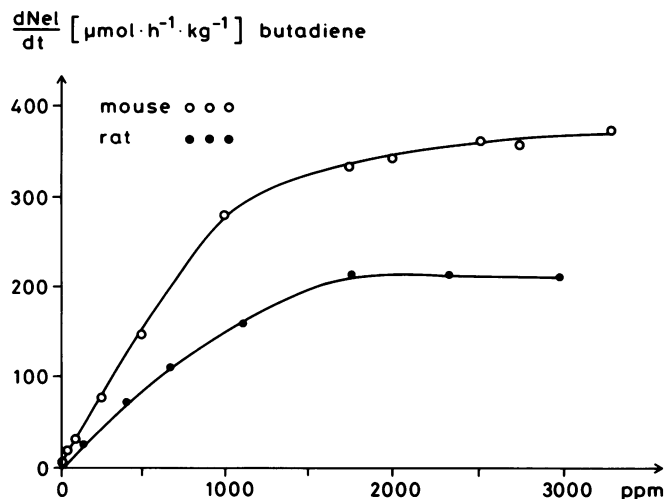


FIGURE 4. Metabolic elimination rates of B6C3F₁ mice (○) and Sprague-Dawley rats (●) dependent on atmospheric concentrations of 1,3-butadiene.

Table 2. Rates of 1,3-butadiene metabolism under conditions of the exposure concentrations applied in two inhalation bioassays with rats (2) and mice (1).

	Exposure concentration, ppm	Amount metabolized, μmole/hr/kg
Rat, Sprague-Dawley (Hazleton Laboratories, Europe)	1000	140
	8000	220
Mouse, B6C3F ₁ (NTP Bioassay Program)	625	165
	1250	300

Up to ambient concentrations of about 1000 ppm, the metabolic elimination of 1,3-butadiene is proportional to the exposure concentration in mice and rats. Above 1000 ppm the saturation kinetics of 1,3-butadiene metabolism become apparent in both species. A comparison of the metabolic elimination rates of both species at different exposure concentrations reveals that the metabolic elimination rate of 1,3-butadiene in mice is about twice that in rats, both under conditions of low- and high-exposure concentrations. Based on Figure 4, the actual rates of 1,3-butadiene metabolism in both species can be calculated for the exposure concentrations used in the two long-term bioassays with rats (2) and mice (1). Such values may be derived under the assumption that 1,3-butadiene metabolism in mice and rats remains constant during chronic exposure. A comparison of the data (Table 2) shows that under the particular bioassay conditions, mice metabolized only about 35% more 1,3-butadiene than rats.

Inhalation Pharmacokinetics of 1,2-Epoxybutene-3

Investigations of inhalation pharmacokinetics of 1,2-epoxybutene-3 revealed major differences in this

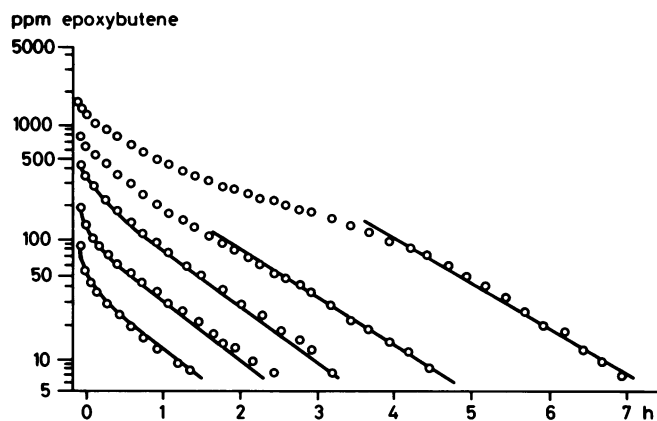


FIGURE 5. Time course of 1,2-epoxybutene-3 concentrations in the gas phase of a closed 6.4-L desiccator jar chamber occupied by eight B6C3F₁ mice. Individual experiments with different initial 1,2-epoxybutene-3 concentrations between 100 and 2000 ppm.

Table 3. Pharmacokinetic parameters for distribution and metabolism of inhaled 1,2-epoxybutene-3 in mice and rats related to 1 kg body weight.^a

Parameter	Mouse	Rat	Dimension
$k_{12}V_1$	33,500	13,800	mL/hr
k_{21}	0.79	0.37	hr ⁻¹
K_{eqa}	42.5	37	—
K_{stb}	10.2	1.16	—
k_{e1}	2.3	11.5	hr ⁻¹
$Cl_{tot}^{b,c}$	24,900	13,400	mL/hr
Metabolic saturation	500	NO ^d	ppm

^aFor definition of parameters see text and Filser and Bolt (13).

^bCalculated for $\rightarrow \infty$.

^cValid for linear range of metabolism.

^dNot observed up to 5000 ppm.

compounds' metabolism between both species (11,16). When mice were exposed in the closed desiccator jar to different initial concentrations of 1,2-epoxybutene-3 between 100 and 2000 ppm, the decline curves showed a clear saturation behavior of 1,2-epoxybutene-3 metabolism (11) (Fig. 5). At lower concentrations the elimination of 1,2-epoxybutene-3 is directly proportional to its concentration in the gas phase of the system. At higher 1,2-epoxybutene-3 concentrations, the slopes of the concentration time curves decrease, and the saturation of 1,2-epoxybutene-3 metabolism becomes apparent. In contrast to these data, only monoexponential decline curves were observed when rats were exposed to different initial 1,2-epoxybutene-3 concentrations between 10 and 5000 ppm (16).

In rats no indication of the saturation kinetics of 1,2-epoxybutene-3 metabolism could be observed up to exposure concentrations of 5000 ppm, whereas in mice the saturation of 1,2-epoxybutene-3 metabolism becomes apparent at atmospheric concentrations near 500 ppm. The pharmacokinetic parameters for distribution and metabolism of 1,2-epoxybutene-3 were determined from the concentration decline curves obtained (11,16) (Table 3). They show that in the lower concentration

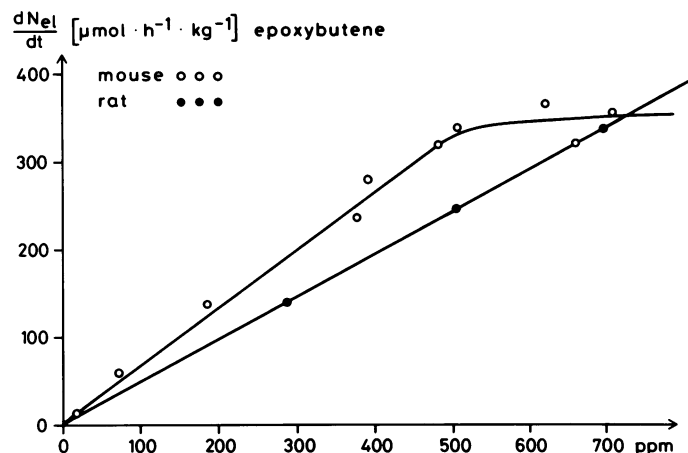


FIGURE 6. Metabolic elimination rates of B6C3F₁ mice (○) and Sprague-Dawley rats (●) depending on atmospheric concentrations of 1,2-epoxybutene-3. Metabolic elimination of epoxybutene in rats is linearly depending on the atmospheric concentration of the compound up to 5000 ppm.

range where first-order metabolism applies (up to about 500 ppm), 1,2-epoxybutene-3 is metabolized by mice at higher rates than rats (metabolic clearance per kilogram body weight, mice: 24,900 mL/hr; rats: 13,400 mL/hr). Under these conditions the steady-state concentration of 1,2-epoxybutene-3 in the mouse was calculated to be about 10 times that in the rat. The calculated maximal metabolic rate for 1,2-epoxybutene-3 was 350 $\mu\text{mole/hr/kg}$ in mice and $> 2600 \mu\text{mole/hr/kg}$ in rats. A comparison of the metabolic elimination rates of 1,2-epoxybutene-3 in both species (calculated for conditions of exposure in an open ($V_1 \rightarrow \infty$) system) reveals (Fig. 6) that at lower exposure concentrations mice show a higher metabolic rate for 1,2-epoxybutene-3 than rats. The metabolic elimination of inhaled 1,2-epoxybutene-3 in rats is linearly dependent on the atmospheric concentration, at least up to exposure concentrations of about 5000 ppm, whereas in mice the saturation of 1,2-epoxybutene-3 metabolism becomes apparent at about 500 ppm. Therefore, with increasing exposure concentration the metabolic capacity for 1,2-epoxybutene-3 becomes rate limiting in mice, but not in rats.

Exhalation of 1,2-Epoxybutene-3

Exhalation of 1,2-epoxybutene-3 into the atmosphere of the closed exposure system is observed when mice or rats are exposed to 1,3-butadiene (11,16). In both experiments 1,3-butadiene concentrations were maintained above 2000 ppm, which ensured that the metabolism of 1,3-butadiene proceeded under saturation conditions. Remarkable differences are obvious between both species (Fig. 7). 1,2-Epoxybutene-3 exhaled by rats reaches a plateau concentration of about 4 ppm, whereas its exhalation by mice leads to an increase in concentration, until a peak concentration of about 10 ppm in the system is reached after 10 hr. The subsequent decline in the atmospheric epoxide concentration in the experiment

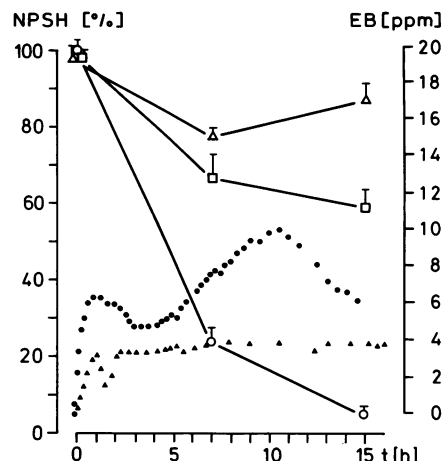


FIGURE 7. Decrease of hepatic NPSH content in butadiene-exposed mice (○, B6C3F₁) and rats (△, Sprague-Dawley; □, Wistar); upon exposure to 1,3-butadiene at concentrations (> 2000 ppm) that cause maximal metabolism to 1,2-epoxybutene-3 for both species. Percent of the corresponding controls are shown. Values represent six mice or three rats \pm SD. For comparison the time courses are included of 1,2-epoxybutene-3 concentrations (EB) exhaled into the gas phase of the closed exposure system (6.4-L) by two Sprague-Dawley rats (▲) or six B6C3F₁ mice (●) under similar experimental conditions.

with mice is due to a decrease in 1,3-butadiene metabolism. From about 12 hr onward, mice show signs of acute toxicity, and lethality occurs when the 1,3-butadiene exposure is prolonged over 15 hr. No toxicity was observed in rats using the same protocol.

The differences in 1,2-epoxybutene-3 exhalation and in the toxicity of 1,3-butadiene between mice and rats can be easily explained by the differences in pharmacokinetics. Since the metabolic elimination of 1,2-epoxybutene-3 in mice is a saturable process, the concentration of 1,2-epoxybutene-3 metabolically generated from 1,3-butadiene gradually increases in the animal (under saturation conditions of 1,3-butadiene metabolism). Because exhalation of a volatile compound is proportional to its concentration in the animal, this also results in an increase in 1,2-epoxybutene-3 concentration in the atmosphere of the closed-exposure system. The final decline in 1,3-butadiene metabolism in the mouse experiment (Fig. 7), which is associated with a reduction in epoxide exhalation, can be attributed to a toxic action of 1,2-epoxybutene-3. This is supported by the fact that at the end of the 15-hr exposure period the hepatic nonprotein sulfhydryl content is reduced to about 4% of that of nonexposed animals. Under similar conditions of 1,3-butadiene exposure in rats, the hepatic nonprotein sulfhydryl content shows no major depletion.

Depletion of Hepatic Nonprotein Sulfhydryl Content

After the inhalation exposure of mice (B6C3F₁) and rats (Sprague-Dawley, Wistar) remarkable species differences in the extent and time course of depletion of

ferences in the extent and time course of depletion of liver nonprotein sulfhydryl (NPSH) content are obvious (Fig. 7). Exposure of mice to concentrations of >2000 ppm 1,3-butadiene resulted in a progressive depression of hepatic NPSH to a value of about 20% after 7 hr and a practically total depletion of hepatic NPSH after 15 hr. In rats the hepatic NPSH content was depleted to values between 65% (Wistar) and 80% (Sprague-Dawley) after 7 hr, but they showed no major changes when exposure to 1,3-butadiene was continued for 8 hr. At the end of the 15-hr exposure to 1,3-butadiene, mice showed signs of acute toxicity. Neither Wistar or Sprague-Dawley rats showed signs of toxicity after a 15-hr exposure to 1,3-butadiene.

In addition to the higher production rate of epoxybutene from 1,3-butadiene in mice, metabolism of 1,2-epoxybutene-3 is saturable in mice (B6C3F₁) but not in rats (Sprague-Dawley). This leads, at high exposure concentrations of 1,3-butadiene, to a continuous accumulation in mice of 1,2-epoxybutene-3, which can be traced in the exhaled air of the animals. A comparison of the time course of hepatic NPSH depletion with the time course of 1,2-epoxybutene-3 concentrations in the closed system (Fig. 7), obtained under similar experimental conditions, shows that both parameters are related to each other. After an initial moderate decline in rats, the hepatic NPSH levels show no major changes and the 1,2-epoxybutene-3 exhalation remained constant until exposure to 1,3-butadiene was ended. For mice an increase in 1,2-epoxybutene-3 exhalation can be observed until after about 10 hr of exposure to 1,3-butadiene, then the hepatic NPSH levels are depleted to about 10% of their initial values. The subsequent reduction in 1,2-epoxybutene-3 exhalation by mice and the toxicity observed when exposure to 1,3-butadiene is continued can be attributed to this effect.

1,2-Epoxybutene-3 is conjugated with glutathione (4) and can be metabolized by glutathione-S-transferase (5). Hepatic NPSH depletion by 1,3-butadiene may thus be regarded as the combined result of spontaneous and enzyme-mediated conjugation of reactive 1,3-butadiene intermediates with glutathione (20).

With regard to the chemical stability of reactive 1,3-butadiene intermediates (21) and their accumulation in the mouse, (8,11), it seems reasonable to assume that reductions of hepatic NPSH in mice may reflect the situation in target organs like the lung and heart.

Alkylation of Nuclear Proteins and DNA

After exposure of mice (B6C3F₁) or rats (Wistar) to (¹⁴C)-butadiene, radioactivity was covalently bound to liver nucleoprotein fractions and to total liver DNA of both species (Fig. 8). This shows that reactive 1,3-butadiene metabolites alkylate liver nucleoproteins and DNA under conditions of exposure *in vivo*. Covalent binding of radioactivity to liver nucleoproteins of mice was about twice as high as in rats. This shows that, in parallel to the higher metabolic rate of butadiene in the mouse, the formation rate of reactive protein-binding

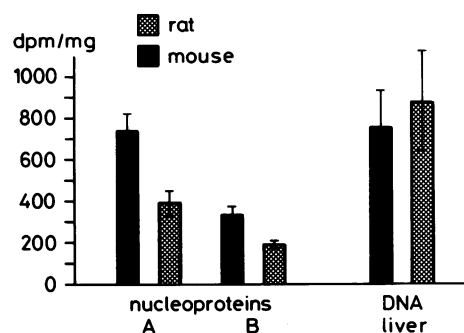


FIGURE 8. Radioactivity in nucleoproteins and DNA of mouse (B6C3F₁) and rat (Wistar) liver after exposure of the animals to (1,4-¹⁴C) 1,3-butadiene. (A) Early eluting nucleoproteins; (B) late eluting nucleoproteins. Radioactivity uptake per kilogram body weight was about 2.7 mCi for mice and rats.

metabolites is proportionally increased in this species. Comparable amounts of (¹⁴C)-butadiene-derived radioactivity were associated with the liver DNA of both species, although 1,3-butadiene is metabolized in rats and mice at different rates. It is not clear to which extent the total radioactivity bound to liver DNA represents DNA alkylation at specific DNA bases or the metabolic incorporation into the physiological nucleosides. Recently the formation in DNA of 7-(2-hydroxy-3-buten-1-yl) guanine and of 7-(1-hydroxy-3-buten-2-yl) guanine has been demonstrated after chemical reaction of epoxybutene with DNA *in vitro* (22). Although this supports the assumption that 1,2-epoxybutene-3, as the major reactive intermediate, covalently binds to DNA, we cannot rule out that diepoxybutane and/or 3,4-epoxy-1,2-butanediol contribute to this effect. Further data on the chemical nature and possible individual differences of specific DNA adducts between rat and mouse are necessary.

Conclusions

These investigations revealed that differences in species susceptibility to inhaled 1,3-butadiene between rats and mice are related to differences in butadiene metabolism. We conclude that, in addition to the higher production rate of 1,2-epoxybutene-3 from 1,3-butadiene in mice versus rats, limited detoxification and thus accumulation of this primary reactive intermediate may be a major determinant for the higher susceptibility of mice to 1,3-butadiene-induced carcinogenesis.

This view is supported by observations that, under exposure to high concentrations of 1,3-butadiene, exhalation of 1,2-epoxybutene-3 by mice is two to three times that of rats, hepatic NPSH content is almost completely depleted, and considerably higher blood levels of 1,2-epoxybutene-3 (two to five times) and diepoxybutane (up to three times) occur in mice (8).

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