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Species differences in butadiene metabolism between mice and rats evaluated by inhalation pharmacokinetics

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Abstract. Metabolism of 1,3-butadiene to 1,2-epoxybutene-3 in rats follows saturation kinetics. Comparative investigation of inhalation pharmacokinetics in mice also revealed a saturation pattern. For both species "linear" pharmacokinetics apply at exposure concentrations below 1000 ppm 1,3-butadiene; saturation of butadiene metabolism is observed at atmospheric concentrations of about 2000 ppm.

For mice metabolic clearance per kg body weight in the lower concentration range where first order metabolism applies was 7300 $ml \times h^{-1}$ (rat: 4500 $ml \times h^{-1}$). Maximal metabolic elimination rate (V_{max}) was 400 $\mu mol \times h^{-1} \times kg^{-1}$ (rat: 220 $\mu mol \times h^{-1} \times kg^{-1}$). This shows that 1,3-butadiene is metabolized by mice at higher rates compared to rats.

Based on these investigations, the metabolic elimination rates of butadiene in both species were calculated for the exposure concentrations applied in two inhalation bioassays with rats and with mice. The results show that the higher rate of butadiene metabolism in mice when compared to rats may only in part be responsible for the considerable difference in the susceptibility of both species to butadiene-induced carcinogenesis.

Key words: 1,3-Butadiene – Inhalation – Pharmacokinetics – Species differences – Metabolism

Introduction

1,3-Butadiene is used in the production of synthetic rubber, thermoplastic resins and as a comonomer for plasticizers (Kirk-Othmer Enzyclopedia of Chemical Technology 1979).

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 (Malvoisin et al. 1979; Malvoisin and Roberfroid 1982; Bolt et al. 1983).

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 (Malvoisin and Roberfroid 1982; Malvoisin et al. 1982).

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When rats are exposed to 1,3-butadiene, exhalation by the animals of 1,2-epoxybutene-3 is observed (Bolt et al. 1983; Filser and Bolt 1984). This shows that butadiene is biotransformed in experimental animals to a reactive epoxide intermediate which is systemically available to the organism.

Whereas butadiene was weakly carcinogenic in an inhalation bioassay in rats (Sprague Dawley; Hazleton Laboratories Europe 1981) a recent long-term inhalation study in mice ($B_6C_3F_1$, Huff et al. 1985) revealed a considerably higher carcinogenic activity of butadiene in this species, with malignant neoplasms occurring at multiple sites. Recent data on metabolism of butadiene in microsomal incubates from liver of mouse, rat, monkey and man (Schmidt and Loeser 1985) suggest considerable species differences in metabolism of this compound.

The purpose of the present study was to investigate whether the higher susceptibility of mice to butadiene (compared to rats) was due to quantitative differences in butadiene metabolism between these two species. Therefore, the pharmacokinetics of distribution and metabolism of 1,3-butadiene in mice are described.

A comparison of these results with the pharmacokinetic parameters of butadiene in rats was based on a previous study (Bolt et al. 1984).

Materials and methods

Animals. Male B6C3F1 mice (Zentralinstitut für Versuchstierzucht, Hannover, FRG) with a body weight of 25–30 g were used. Previously published procedures (Filser and Bolt 1983) were used to relate the kinetic parameters to 1 kg body weight.

Pretreatment. In some experiments dithiocarb as a metabolic inhibitor (diethyldithiocarbamate, Merck) was administered at a single dose of 300 mg/kg weight, IP, in saline (solution of 50 mg dithiocarb/ml saline), 45 min prior to the experiment.

Gas uptake and kinetic studies. Usually eight mice were placed in a closed 6.4 l desiccator jar chamber, equipped with 135 g soda lime for CO_2 absorption and an oxygen supply. The animals were exposed to initial concentrations between 10 ppm and 5000 ppm 1,3-butadiene. Concentration changes in the gas phase of the system were measured by gas chromatography after injection of butadiene into

the system (animals with and without dithiocarb pretreatment) or after IP administration of butadiene to the animals (IP studies: three mice, 2.8 l dessiccator, 63 g soda lime). Concentration changes were recorded for the time periods indicated in the figures. 1,3-Butadiene (99.0% pure) was purchased from Messer-Griesheim, Düsseldorf, FRG.

Kinetic parameters were determined based on a two-compartment, open pharmacokinetic model developed by Filser and Bolt (1983). This model implies a one-compartment description of the experimental animal. The gas phase in the desiccator with volume V_1 represented compartment 1 (Cp 1) the animals with volume V_2 compartment 2 (Cp 2).

Gas chromatography. A Shimadzu gas chromatograph (GC-8A equipped with gas sampler MGS-4) with a 5-ml gas sample loop and an FID was used. Butadiene was chromatographically separated on a 1-m stainless steel 1/8 Porapak Q GC column (80–100 mesh) at an oven temperature of 135 °C. The FID temperature was 200 °C. Gas flow rates were as follows: carrier gas (N_2), 60 ml/min; hydrogen, 25 ml/min; synthetic air, 240 ml/min. Under these conditions the retention time for butadiene was 1.3 min.

Results

Starting from different initial concentrations between 10 ppm and 5000 ppm, the time-dependent decline of butadiene in the exposure system was investigated. The decline curves (Fig. 1) observed in these experiments became flatter at higher exposure concentrations, indicating a saturable elimination. Below concentrations of about 1000 ppm, the elimination of butadiene can be described by a first order process. At higher atmospheric concentrations saturation kinetics become apparent; V_{max} has been calculated to be 400 $\mu mol \times h^{-1} \times kg^{-1}$ (Table 1).

To analyze the initial process of equilibration between uptake, exhalation and metabolism of butadiene, which is determined by the rate constants of equilibration k_{12} and k_{21} and the rate constant for first order metabolic elimination (k_{el}) (Filser and Bolt 1981, 1983), additional experiments were performed.

The equilibration of butadiene between gas phase and animal compartment was measured after pretreatment of the animals with dithiocarb as a metabolic inhibitor. Following a distribution-dependent decline of butadiene in the gas phase of the system, a constant equilibrium was achieved (Fig. 2). As described for rats (Bolt et al. 1984), metabolism of butadiene in mice was inhibited by pretreatment with dithiocarb. From this experiment the coefficient of static distribution, K_{eq}, was calculated according to Filser and Bolt (1979).

As already observed in rats (Bolt et al. 1984) metabolic elimination of butadiene in mice under inhalation conditions was practically limited by the uptake rate of the compound from the gas phase into the animal organism. Hence, the rate constant for first-order metabolic elimination (k_{el}) of butadiene could not be obtained from the inhalation experiments alone with sufficient accuracy. To overcome this difficulty butadiene was administered IP to the animals (see Bolt et al. 1984). Figure 3 shows that butadiene, administered IP to mice, is partly exhaled. From the

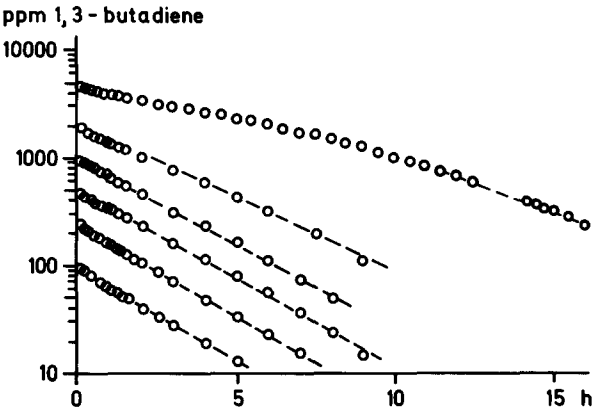

Fig. 1. Time-course of 1,3-butadiene concentrations in the gas phase of a closed 6.4-l desiccator jar chamber, occupied by eight animals (B6C3F1 mice untreated). Individual experiments with different initial butadiene concentrations show a first order decline at concentrations below 1000 ppm

Table 1. Pharmacokinetic parameters for distribution and metabolism of 1,3-butadiene in mice and rats (Bolt et al. 1984) related to 1 kg body weight [a]

Parameter	Mouse	Rat	Dimension
$k_{12}V_1$	10280	5750	$ml \cdot h^{-1}$
k_{21}	3.2	2.5	h^{-1}
K_{eq}	2.7	2.3	–
K_{st} [b]	1.0	0.5	–
k_{el} [c]	7.6	8.8	h^{-1}
Cl_{tot} [a,b]	7300	4500	$ml \cdot h^{-1}$
V_{max}	400	220	$\mu mol \cdot h^{-1} \cdot kg^{-1}$

[a] In order to compare $k_{12}V_1$ and k_{21} (which depend on the experimental conditions) between different experiments and between rats and mice, the figures were standardized, according to the formulae of Filser and Bolt (1983), to theoretical conditions of $n = 2$ and $V_2 = 1000$ ml.

[b] Calculated for $V_1 \rightarrow \infty$

[c] Valid for linear range (up to 1000 ppm for both species)

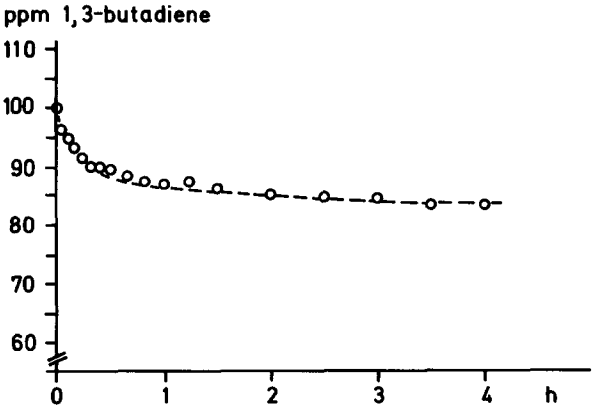

Fig. 2. Time-course of 1,3-butadiene concentrations in the gas phase of a 6.4-l desiccator jar champer, occupied by eight B6C3F1 mice pretreated with dithiocarb. The data were fitted with a calculated equilibration curve *(dotted line)*

biexponential concentration-time course (together with K_{eq} from experiments with inhibited metabolism) the rate constants of exhalation (k_{21}), invasion (k_{12}) and the rate constant of first-order metabolic elimination (K_{el}) could be calculated according to the mathematical procedures introduced by Bolt et al. (1984).

From the pharmacokinetic parameters thus obtained (see Table 1) the concentration-time courses of butadiene in the different experiments were computed and compared with the actual experimental data (Figs. 1–3, dashed lines). It appeared that these calculated concentration time curves

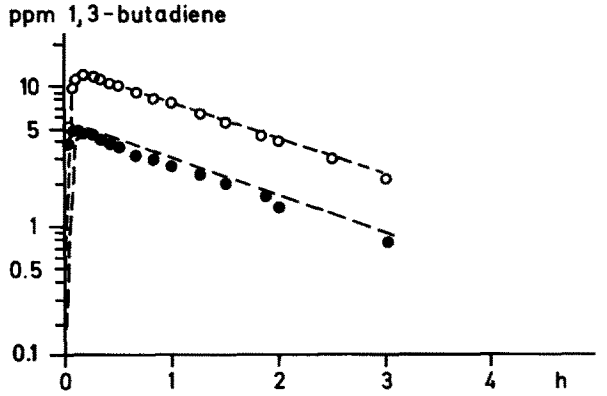

Fig. 3. Time-course of 1,3-butadiene concentrations in a 2.8-l desiccator jar chamber occupied by three animals after IP injection (at t=0) of the compound. Injection of butadiene at two different doses of 350 *(closed circles)* and 900 *(open circles)* nl gas/g body weight, and fittings with "calculated" curves *(dotted lines)*

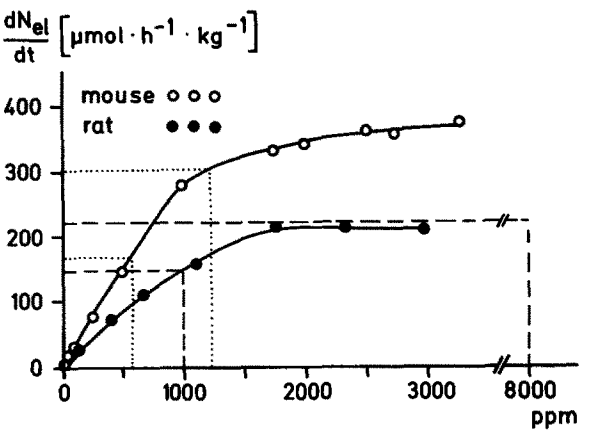

Fig. 4. Metabolic elimination rates per kg body weight (dN_{el}/dt) of mice *(open circles)* and rats *(closed circles)* depending on atmospheric concentrations of 1,3-butadiene. Calculations for inhalation conditions from an "open" ($V_I \rightarrow \infty$) atmosphere (see Filser and Bolt 1981). The rates of butadiene metabolism under conditions of the exposure concentrations applied in two inhalation bioassays with rats *(dotted lines)* and mice *(points*; see Text) were taken from this figure

satisfactorily fitted the experimental data. This shows that the underlying pharmacokinetic model (Filser and Bolt 1983) was consistent with the actual kinetic behaviour of butadiene in mice.

From the individual experiments the metabolic elimination rates of butadiene were calculated for conditions of exposure in an "open" ($V_I \rightarrow \infty$) exposure system (Filser and Bolt 1981); these rates (see Fig. 4) were compared with the data already available for rats (Bolt et al. 1984).

Figure 4 shows that the metabolic elimination of butadiene in mice is proportional to the exposure concentration up to ambient concentrations of about 1000 ppm butadiene. Above 1000 ppm saturation kinetics of butadiene metabolism become visible in this species. A comparison of the metabolic elimination rates of both species at different exposure concentrations reveals that the metabolic elimination rate in mice is about twice that in rats, both under conditions of low and high exposure concentrations.

In Table 1 the pharmacokinetic parameters for butadiene in mice are compared with those previously determined in rats (Bolt et al. 1984).

This comparison reveals that the numerical values for the (standardized) rate constant of exhalation (k_{21}), the rate constant for (first-order) metabolic elimination (k_{el}) and the coefficient of static distribution (K_{eq}) are in about the same range for mice and rats.

The rate constant of invasion (k_{12}) in mice is about twice that in rats. Thus, for the partial process of invasion mice show about twice the clearance ($k_{12} \times V_I$) compared to rats. Together with a similar k_{21} in both species, this results in a twofold higher value for the coefficient of dynamic distribution (K_{st}) in mice. From K_{st} it follows that for exposures under conditions were first-order elimination applies the steady state concentration of butadiene in the body of mice is about twice that in rats.

Discussion

According to Anderson (1981) two major factors may limit the rate of metabolism of volatile xenobiotics which have a high affinity to the metabolizing enzyme system. At high exposure concentrations the enzyme capacity limits the maximal metabolic rate (V_{max}). At low exposure concentrations the availability of the compound for the metabolizing enzymes is often limited by physiological parameters, e.g. respiration frequency, lung and liver perfusion.

Based on this concept, the increased metabolic elimination rate for butadiene in mice, compared to rats, under conditions of low exposure concentrations may reasonably be attributed to the higher respiration frequency, (Phalen 1983), and a higher lung and liver perfusion in this species. This view is corrobarated by the higher clearance of uptake of butadiene ($k_{12} \times V_I$) in mice, resulting in about twice the concentration of butadiene in the animal compartment (Cp 2).

Under conditions of metabolic saturation at high exposure concentrations the higher metabolic capacity of mice, which is about twice that in rats based on the body weight, (Walker 1978) becomes prominent.

Our results show that in principle butadiene is metabolized by mice (B6C3F1) at about twice the rate of rats (Sprague Dawley). Similar species differences have been observed for metabolic elimination of vinyl chloride (Buchter et al. 1978) and perchloroethylene (Schumann et al. 1980; Bolt and Link 1980).

When butadiene metabolism was measured in liver postmitochondrial preparations of B6C3F1-mice and Sprague-Dawley rats, the rate of formation of 1,2-epoxybutene-3 was about 2–3 times higher with liver prepara-

tions, but 5–6 times higher with lung preparations of mice (Schmidt and Löser, 1985). These authors suggested that the higher formation rate of the reactive intermediate epoxide (1,2-epoxybutene-3) in mice, compared to rats, especially in the lung, could be responsible for the differences in the susceptibility of both species to butadiene.

Based on our investigations the actual rates of butadiene metabolism in both species can be calculated for the exposure concentrations utilised in the two long-term bioassays with rats (Sprague-Dawley; Hazleton Laboratories Europe 1981) and mice (B6C3F1; Huff et al. 1985; see Fig. 4). Such values may be derived under the assumption that butadiene metabolism in mice and rats remains constant during chronic exposure.

A comparison of the data shows that under the particular bioassay conditions mice (study of Huff et al. 1985) metabolized only about 35% more butadiene than rats (study of Hazleton Laboratories Europe 1981), both at the respective low and high doses utilised.

This suggests that the particularly pronounced susceptibility of mice to butadiene-induced carcinogenicity may only in part be attributed to a higher overall metabolism rate of butadiene in this species.

It should be noted, however, that this generalized view does not take into account differences in metabolism at local target sites, and differences in inactivation of butadiene monoxide (1,2-epoxybutene-3). In addition, a possible involvement of the di-epoxide (diepoxybutane), which is a bifunctional alkylating agent (van Duuren et al. 1963) in the metabolism of 1,3-butadiene, remains to be elucidated.

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References

Andersen ME (1981) Saturable metabolism and its relationship to toxicity. CRC Crit Rev Toxicol 10: 105–150

Bolt HM, Link B (1980) Zur Toxikologie von Perchloräthylen. Verh Dtsch Ges Arbeitsmed 20: 463–470

Bolt HM, Schmiedel G, Filser JG, Rolzhäuser HP, Lieser K, Wistuba D, Schurig V (1983) Biological activation of 1,3-butadiene to vinyl oxirane by rat liver microsomes and expiration of the reactive metabolite by exposed rats. J Cancer Res Clin Oncol 106: 112–116

Bolt HM, Filser JG, Störmer F (1984) Inhalation pharmacokinetics based on gas uptake studies V. Comparative pharmacokinetics of ethylene and 1,3-butadiene in rats. Arch Toxicol 55: 213–218

Buchter A, Bolt HM, Filser JG, Goergens HW, Laib RJ, Bolt W (1978) Pharmakokinetik und Carcinogenese von Vinylchlorid. Arbeitsmedizinische Risikobeurteilung. Verh Dtsch Ges Arbeitsmed 18: 111–115

Filser JG, Bolt HM (1979) Pharmacokinetics of halogenated ethylenes in rats. Arch Toxicol 42: 123–136

Filser JG, Bolt HM (1981) Inhalation pharmacokinetics based on gas uptake studies I. Improvement of kinetic models. Arch Toxicol 47: 279–292

Filser JG, Bolt HM (1983) Inhalation pharmacokinetics based on gas uptake studies IV. The endogenous production of volatile compounds. Arch Toxicol 52: 123–133

Filser JG, Bolt HM (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

Hazleton Laboratories Europe (1981) "1,3-butadiene. Inhalation teratogenicity study in the rat", Final report and addendum No. 2788–522/3, Hazleton Labs., Harrowgate HG3 1 PY, England

Huff JE, Melnick RL, Solleveld HA, Haseman JK, Powers M, Miller RA (1985) Multiple organ carcinogenicity of 1,3-butadiene in B6C3F$_1$ mice after 60 weeks of inhalation exposure. Science 227: 548–549

Kirk-Othmer Enzyclopedia of Chemical Technology (1979), Wiley, New York, ed. 3, vol. 4, pp 313–337

Malvoisin E, Lhoest G, Poncelet F, Roberfroid M, Mercier M (1979) Identification and quantitation of 1,2-epoxybutene-3 as the primary metabolite of 1,3-butadiene. J Chromatogr 178: 419–425

Malvoisin E, Mercier M, Roberfroid M (1982) Enzymic hydration of butadiene monoxide and its importance in the metabolism of butadiene. Adv Exp Med Biol 38A: 437–444

Malvoisin E, Roberfroid M (1982) Hepatic microsomal metabolism of 1,3-butadiene. Xenobiotica 12: 137–144

Phalen RF (1983) Animal models. In: Inhalation studies formation and techniques, CRC Press Inc., Florida, pp 211–241

Schmidt U, Loeser E (1985) Species differences in the formation of butadiene monoxide from 1,3-butadiene. Arch Toxicol 57: 222–225

Schumann AM, Quast JF, Watanabe PG (1980) The pharmacokinetics and macromolecular interactions of perchlorethylene in mice and rats as related to oncogenicity. Toxicol Appl Pharmacol 55: 207–219

Van Duuren BL, Nelson N, Orris L, Palmes ED, Schmitt FL (1963) Carcinogenicity of epoxides, lactons and peroxy compounds. J Natl Cancer Inst 31: 41–55

Walker CH (1978) Species differences in microsomal monooxygenase activity and their relationship to biological half-lives. Drug Metab Rev 7: 295–323

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