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Inhalation pharmacokinetics based on gas uptake studies

V. Comparative pharmacokinetics of ethylene and 1,3-butadiene in rats

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Abstract. The pharmacokinetics of ethylene and 1,3-butadiene were studied in male Sprague-Dawley rats by use of a closed inhalation chamber system. Both compounds showed saturable metabolism when untreated rats were used. "Linear" pharmacokinetics applied at exposure concentrations below 800 ppm ethylene and below 1,000 ppm 1,3-butadiene. A constant elimination rate, indicative of metabolic saturation, occurred at concentrations higher than 1,000 ppm ethylene or 1,500 ppm 1,3-butadiene. Pretreatment with aroclor 1254 (polychlorinated biphenyls) increased V_{max} for both compounds. For 1,3-butadiene, no saturation of metabolic capacity was observed with exposure concentrations up to 12,000 ppm when the rats were pretreated with aroclor 1254. A comparison with previous studies on ethane and *n*-pentane suggested that introduction of a double bond into a saturated aliphatic hydrocarbon increased the rate of metabolism under conditions in vivo.

Key words: Ethylene – 1,3-Butadiene – Inhalation – Pharmacokinetics

Introduction

The olefinic hydrocarbons ethylene and 1,3-butadiene are transformed to epoxides which are reactive (Jones and Mackrodt 1983) and carcinogenic (Snellings et al. 1981; Dunkelberg 1982; ECETOC 1982; IARC 1976). Rat liver microsomes (in presence of NADPH and O_2) catalyze formation of ethylene oxide from ethylene (Schmiedel et al. 1983) and of butadiene monoxide (vinyl oxirane; 1,2-epoxybutene-3) from 1,3-butadiene (Malvoisin et al. 1979; Malvoisin and Roberfroid 1982; Bolt et al. 1983). Both optical enantiomers (R and S) of butadiene monoxide are formed (Bolt et al. 1983). The epoxides then undergo further enzymic transformations (Calleman et al. 1978; Malvoisin et al. 1982). Evidence from the chemical reactivities suggests formation of specific alkylation products with DNA, preferentially at N-7 of guanine: 7-(2-hydroxyethyl)-guanine (Ashby et al. 1982) and the isomeric hydroxybutenylguanines 7-(2-hydroxy-3-buten-1-yl)guanine and 7-(1-hydroxy-3-buten-2-yl)guanine (Citti et al. 1984) have been suggested as the major alkylation products of ethylene oxide and butadiene monoxide, respectively.

Recently, we have demonstrated exhalation of ethylene oxide by rats exposed to ethylene (Filser and Bolt 1983a). Similarly, on exposure to 1,3-butadiene exhalation of butadiene monoxide was observed (Bolt et al. 1983). A quantitative treatment of metabolism of the olefinic hydrocarbons to their reactive intermediates has considerable importance for risk estimations (Bolt and Filser 1984). Hence, detailed pharmacokinetic studies are needed.

The present paper describes the pharmacokinetics of distribution and metabolism of both ethylene and 1,3-butadiene. For this purpose, previously developed models for gas uptake pharmacokinetics (Filser and Bolt 1979, 1981; Hallier et al. 1981; Bolt et al. 1981; Filser and Bolt 1983b) were utilised. A closed desiccator jar chamber was used in which the animals were housed throughout the experiment; the time course of ethylene or butadiene in this closed atmosphere was followed by gas chromatography.

To allow direct comparison of the results with those obtained with the alkanes ethane and *n*-pentane which were studied earlier (Filser et al. 1983), we followed, in general, the outline of this previous study.

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Materials and methods

Animals. Male Sprague-Dawley rats (Ivanovas, Kissleg, FRG) of 150–280 g were used. Previously published formulae (see Appendix Filser and Bolt 1983b) were used to relate the kinetic parameters to 1 kg body weight. In general, two rats were taken for an experiment.

Pretreatments. In some experiments dithiocarb as a metabolic inhibitor (diethyldithiocarbamate, Merck) was given at a single dose of 200 mg/kg, IP, in saline (solution of 50 mg dithiocarb/ml saline), 30 min prior to the experiment.

Aroclor 1254 (polychlorinated biphenyls) was administered at a single dose of 500 mg/kg (IP, in oil, 200 mg/ml) 6 days prior to the experiment.

Gas uptake and kinetic studies. Usually two animals were placed in a 6.4-l desiccator jar chamber, equipped with 135 g soda lime (or as indicated in the Results section). When differing experimental conditions were applied, quantitative corrections were done as previously described (Filser and Bolt 1983b).

Concentration changes in the gas phase of the system were recorded for the time periods indicated in the figures.

Ethylene (99.95% pure) and 1,3-butadiene (99.0% pure) were purchased from Messer-Griesheim, Düsseldorf, FRG.

Kinetic parameters were determined based on the two compartment open pharmacokinetic model developed previously (Filser and Bolt 1983b). This model implies a one-compartment description of the experimental animal. For ethylene and butadiene this model adequately fitted the experimental data.

Gas chromatography. A Fractovap gas chromatograph with a 5-ml gas sample loop and an FID was used. Ethylene was chromatographically separated on aluminium oxide at 180° C as described elsewhere (Filser et al. 1983). Chromatography of 1,3-butadiene was performed on Tenax GC or Carbowax (Bolt et al. 1983).

Results

Uptake of ethylene from the gas phase

Different initial concentrations (between 1 ppm and 6,000 ppm) of ethylene were adjusted in the closed (6.4 l) system which was occupied by two rats. The exponential decline curves (Fig. 1a) obtained in these experiments became flatter at higher concentrations. This has already been observed with halogenated ethylenes (Filser and Bolt 1979) and suggested saturation of metabolizing enzymes at concentrations higher than 800–1,000 ppm.

To analyze the initial process of "equilibration" of the animals with the gas phase which is determined by the microconstants k_{12} and k_{21} (see Filser and Bolt 1979, 1981, 1983b) two experiments (Fig. 1b) were carried out after pretreatment of the animals with dithiocarb (diethyldithiocarbamate) which inhibits the metabolizing cytochrome P-450 system. From these experiments the "equilibrium constant" K_{eq} and the microconstants k_{12} and k_{21} were calculated, as described by Filser and Bolt (1979). All the individual curves in Fig. 1a were then approximated by exponential functions; k_{el} and the remainder of the kinetic parameters being calculated according to Filser and Bolt (1979, 1981, 1983b). Calculations of the metabolic rates (dN_{el}/dt) in these experiments resulted in the curve (open circles) shown in Fig. 4a. This figure indicates that below 800–1,000 ppm ethylene in the atmosphere metabolic elimination proceeds according to a first order rule. Above this range a plateau is found, indicative of zero-order (saturation) elimination.

The mean values from the different experiments resulted in the pharmacokinetic microconstants and parameters listed in Table 1.

Uptake of butadiene from the gas phase

The experiments with butadiene followed the sequence of those described above for ethylene. Figure 2 shows the decline curves determined in the gas phase of the closed chamber obtained both with untreated rats and with dithiocarb-pretreated rats (Fig. 2b).

In general, the decline of butadiene was faster than that of ethylene (Fig. 1a, Fig. 2a). Below 1,000 ppm the exponential declines (Fig. 2a) indicated that a "linear" kinetic model was appropriate. Above this concentration a saturation pattern was observed.

The pharmacokinetic parameters finally obtained (see below) are also included in Table 1.

Calculation of k_{el} for butadiene required additional experiments in which the gas was administered intraperitoneally.

Intraperitoneal application of ethylene and butadiene

After IP administration of ethylene or butadiene (administration into compartment Cp_2: Filser and Bolt 1983b, Fig. 1) the compound appears within seconds in the exhaled air. The concentration curve in the atmosphere of the closed system provides additional insights into the kinetic behaviour. The appropriate mathematical treatment is given in the Apendix.

In the case of *ethylene* all the kinetic parameters (Table 1) could be derived from experiments on inhalation exposure (Fig. 1). From these data, the exhalation curves after IP administration of three different doses of ethylene were predicted. After actually performing the experiments (Fig. 3a), it appeared that the prediction satisfactorily fitted the experimental data. This shows that the model is consistent with the observations.

The microconstant k_{el} for *butadiene* could not be obtained from the inhalation experiments with sufficient accuracy (see Appendix). Hence, the concentration curves observed after IP administration were used to obtain k_{el}. The choice between the two mathematically possible solutions (Eq. 13) was made knowing the value of k_{12} (Eq. 11) from the "inhibition experiments" of Fig. 2b.

Metabolic elimination of ethylene and butadiene

Figure 4 shows the metabolic elimination rates of ethylene (Fig. 4a) and butadiene (Fig. 4b) in rats, calculated from the individual experiments for conditions of exposure in an "open"

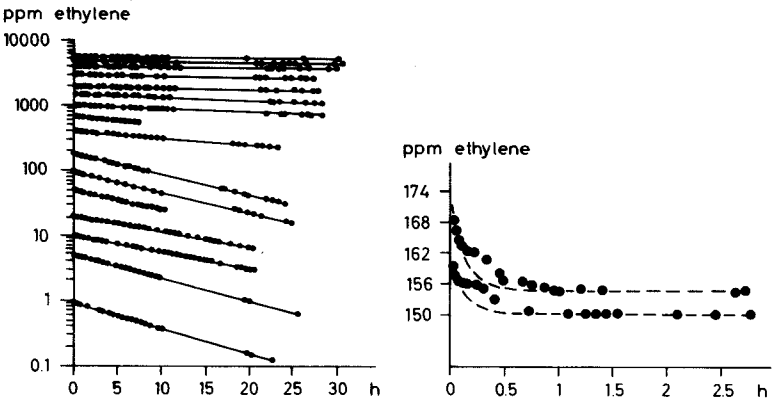

Fig. 1. Time-course of *ethylene* concentrations in the gas phase of a 6.4-l desiccator jar chamber, occupied by two Sprague-Dawley rats. Individual experiments starting from different initial concentrations are shown. (**a**) Left panel: experiments with untreated rats; (**b**) right panel: two experiments with rats pre-treated with dithiocarb to inhibit metabolism. The data were fitted with a (theoretical)-equilibration curve

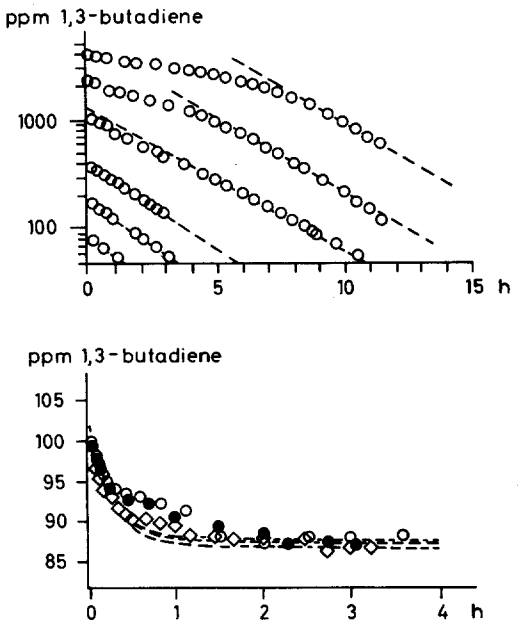

Fig. 2. Time course of *1,3-butadiene* concentrations in the gas phase of 6.4 l desiccator jar chamber, occupied by two Sprague-Dawley rats. (**a**) Top panel: experiments with different initial concentrations (untreated rats) show a first-order decline at concentrations below 1,000 ppm; (**b**) bottom panel: experiments (different symbols) with rats pretreated with dithiocarb and fittings with (theoretical) equilibration curves

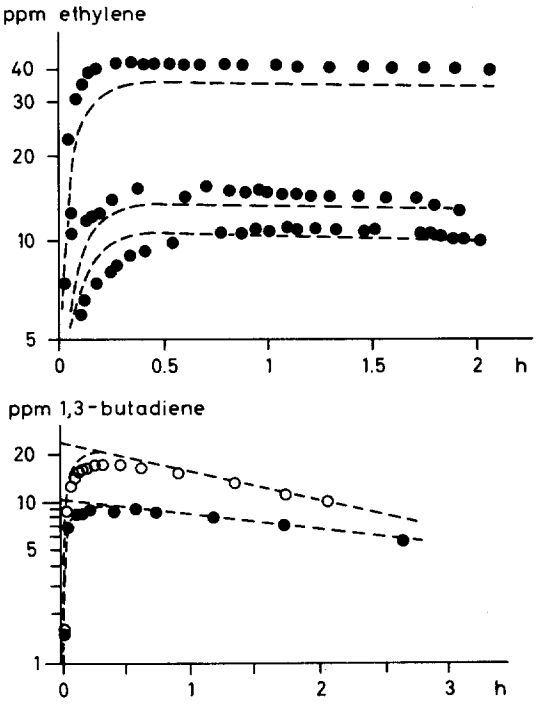

Fig. 3. Concentrations of *ethylene* (**a**) and *1,3-butadiene* (**b**) in the 6.4-l desiccator jar chamber after IP injection (at $t = 0$) of the compounds. **a** (top panel): Injection of ethylene (one animal each) at three different doses of 280, 450, and 1,500 nl gas/g body weight, and fittings with "predicted" curves (see text). **b** (bottom panel): Injection (IP) of 1,3-butadiene; *open circles:* two rats at a dose of 1,100 nl gas/g body weight; *closed circles:* one rat at a dose of 905 nl gas/ml body weight (see text). Extrapolations to C_2 at $t = 0$ are shown ($C_1 = -C_2$; see Appendix)

Table 1. Pharmacokinetic parameters for distribution and metabolism of ethylene and 1,3-butadiene[a]

Compound	Parameter	Value	Dimension
Ethylene	$k_{12}V_1$	3,780	ml × h^{-1}
	k_{21}	5.4	h^{-1}
	K_{eq}	0.70	
	K_{st}[b]	0.50	
	k_{el}[c]	2.1	h^{-1}
	Cl_{tot}[b,c]	1,050	ml × h^{-1}
	V_{max}[d]	8.5	µmol × h^{-1} × kg^{-1}
1,3-Butadiene	$k_{12}V_1$	5,750	ml × h^{-1}
	k_{21}	2.5	h^{-1}
	K_{eq}	2.3	
	K_{st}[b]	0.51	
	k_{el}[c]	8.8	h^{-1}
	Cl_{tot}[b,e]	4,490	ml × h^{-1}
	V_{max}[f]	220	µmol × h^{-1} × kg^{-1}

[a] Experimental conditions: two (theoretical) Sprague-Dawley rats, each 500 g (see Filser and Bolt 1983)
[b] Calculated for $V_1 \rightarrow \infty$
[c] Valid for atmospheric concentrations up to 800 ppm (linear range)
[d] Valid for atmospheric concentrations above 1,000 ppm ethylene (saturation range)
[e] Valid for atmospheric concentrations up to 1,000 ppm butadiene (linear range)
[f] Valid for atmospheric concentrations above 1,500 ppm butadiene (saturation range)

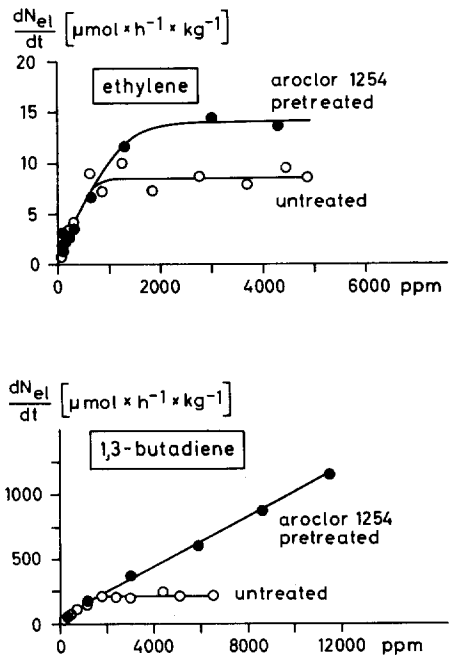

Fig. 4. Metabolic elimination rats (dN_{el}/dt) calculated for different experiments (different data points), depending on atmospheric concentrations of ethylene (**a**, top panel) or 1,3-butadiene (**b**, bottom panel). Calculations for inhalation conditions from an "open" atmosphere, $V_1 \rightarrow \infty$ (see Filser and Bolt 1981)

system ($V_1 \rightarrow \infty$) according to the procedure described by Filser and Bolt (1981). In addition, results obtained after pretreatment with the inducing agent aroclor 1254 (polychlorinated biphenyls) are shown.

These curves (control animals) exhibit a clear saturation pattern; first-order elimination is followed by zero-order elimination at higher ambient concentrations. Pretreatment with aroclor 1254, which leads to an increase in the amount of metabolizing enzymes (cytochrome P-450), increases V_{max} and causes a shift of the "saturation point (Sp)", but leaves the first-order elimination rate unaffected. With butadiene, after aroclor pretreatment, no (apparent) enzyme saturation could be observed within the exposure range studied (up to 12,000 ppm, see Fig. 4b). This can be explained, according to Andersen (1981), by superposition of two different factors which may both be rate-limiting: at lower concentrations (first-order) transport to the enzymic site would determine the metabolic rate in vivo, whereas at higher concentrations the metabolic capacity of the responsible enzymes would limit the maximal turnover (V_{max}). The present data are in full agreement with this concept.

Discussion

On the basis of rat liver microsomal studies in vitro and studies on the chemical reactivities of ethylene oxide and butadiene monoxide (Schmiedel et al. 1983) we have suggested that the lower toxicity of ethylene, compared to butadiene, is due to both a lower alkylating power of ethylene oxide than of butadiene monoxide, and to a lower transformation rate for ethylene than for butadiene. The latter is corroborated by the present data: k_{el} and Cl_{tot} (see Table 1) are considerably higher for butadiene than for ethylene. However, the greatest difference is seen in V_{max} which is 26-fold higher for butadiene than for ethylene. In the light of Andersen's hypothesis (see above) of different factors limiting metabolic rates in vivo, this would suggest that butadiene is in fact a "better substrate" for the metabolizing enzymes than is ethylene.

The available literature on butadiene (Malvoisin et al. 1979, 1982; Malvoisin and Roberfroid 1982) and on ethylene (Segerbäck 1983) agrees that metabolism of both olefins proceeds entirely via primary epoxidation. The complete inhibition by dithiocarb (this study) is also in agreement with the general assumption that cytochrome P-450 is the enzyme system responsible for this primary step.

A series of experiments on ethylene pharmacokinetics has been already published by Andersen et al. (1980). Although their pharmacokinetic model was different from ours in that they used a Michaelis-Menten types of approach to explain the metabolic saturation, both sets of data are principally in agreement. Thus, very similar figures for V_{max} were obtained in both studies.

The present data for ethylene and butadiene may be compared with those for the saturated hydrocarbons ethane and *n*-pentane, which have been previously studied (Filser et al. 1983). Differences in distribution patterns between gas phase and organism of alkanes and alkenes, at first glance, are not visible. The "static" equilibrium (which is reached in absence of metabolism) is very similar for ethane ($K_{eq} = 0.61$) and ethylene ($K_{eq} = 0.70$) as well as for *n*-pentane ($K_{eq} = 2.7$) and butadiene ($K_{eq} = 2.3$). In general, it appears that introduction of a double bond into such aliphatic hydrocarbons enhances the rate of metabolic elimination in vivo. The numerical value of k_{el} is determined by the characteristics of the metabolizing enzymes as well as by the transport to the site of metabolism. k_{el} was 0.73 h^{-1} for ethane, but 2.1 h^{-1} for ethylene; k_{el} for *n*-pentane (5.46 h^{-1}) was also lower than that for butadiene (8.8 h^{-1}). As metabolic elimination (k_{el} and Cl_{tot}) increases with increasing chain length (Frank et al. 1980), the "dynamic" equilibrium (K_{st}) differs greatly from the "static" one (K_{eq}) for both *n*-pentane and butadiene: the "animal compartment", Cp_2, is constantly depleted by metabolic processes. For butadiene, the metabolic rate is consequently largely limited by the influx (k_{12}) of the compound into the organism.

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Appendix

Intraperitoneal administration of volatile compounds

According to the mathematical analysis introduced by Bolt et al. (1981), administration of a volatile compound into the gas phase of the closed exposure system normally results in a biexponential decline, with the intercepts C_1 and C_2 and the first order rate constants λ_1 and λ_2 (λ_1 and λ_2 being negative). In the present case, the first process is visualized in the experiments after metabolic inhibition, which may lead to determination of k_{12}, k_{21} and k_{eq}. As in Eq. (28) of Filser and Bolt (1981), k_{el} is obtained by

$$k_{el} = -\lambda_2 \left(1 + \frac{k_{21}}{k_{12} + \lambda_2}\right). \tag{1}$$

This means that the ratio $k_{12}\,\lambda_2$ has considerable influence on the calculation: when λ_2 is of the same order of magnitude as k_{12}, it is practically impossible to use Eq. (1) for calculation of k_{el}. In this case, the decline in the chamber (λ_2) is primarily influenced by k_{12} (and k_{21}).

The difficulty may be overcome by injection (IP) of the compound. In this case the biexponential concentration curve in the chamber has the form shown in Fig. 3b. Because of

$$y_{1(0)} = C_1 + C_2 = 0 \tag{2}$$

one obtains

$$C_1 = -C_2\,. \tag{3}$$

Because of Eq. (2), Eq. (21) of Bolt et al. (1981) is transformed to

$$\frac{dy_{1(0)}}{dt} = k_{21}\, y_{2(0)}\, \frac{V_2}{V_1}\,, \tag{4}$$

and Eq. (22) of Bolt et al. (1981) is similary transformed to

$$\frac{dy_{2(0)}}{dt} = -(k_{21} + k_{el})\, y_{2(0)}\,. \tag{5}$$

Equation (30) of Bolt et al. (1981) leads to

$$\frac{dy_{1(0)}}{dt} = C_1 (\lambda_1 - \lambda_2) = C_2 (\lambda_2 - \lambda_1) . \quad (6)$$

Introduction of the roots of the fundamental equation [Eqs. (27a) and (27b) of Bolt et al. (1981)] into Eq. (6) leads, with Eq. (4) to

$$C_1 = \frac{- k_{21}\ y_{2(0)}\ V_2}{V_1\ \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4\ k_{12}\ k_{el}}} \quad (7)$$

and

$$C_2 = \frac{+ k_{21}\ y_{2(0)}\ V_2}{V_1\ \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4\ k_{12}\ k_{el}}} . \quad (8)$$

To determine the microconstants (k_{12}, k_{21}, k_{el}) from parameters taken from an experiment as shown in Fig. 5 the following procedure may be followed:

Eqs. (4) and (6) give

$$k_{21} = \frac{V_1 C_2 (\lambda_2 - \lambda_1)}{V_2\ y_{2(0)}} . \quad (9)$$

Addition of Eqs. (27a) and (27b) of Bolt et al. (1981) gives

$$\lambda_1 + \lambda_2 = - (k_{12} + k_{21} + k_{el}) , \quad (10)$$

and, in consequence,

$$k_{12} = - (k_{21} + k_{el} + \lambda_1 + \lambda_2) . \quad (11)$$

Inserting Eq. (11) into Eq. (27a) of Bolt et al. (1981), one finally obtains

$$k_{el}^2 + (k_{21} + \lambda_1 + \lambda_2)\ k_{el} + \lambda_1 \lambda_2 = 0 \quad (12)$$

with the two solutions (1, 2)

$$k_{el(1,2)} = \frac{1}{2}\left[- (k_{21} + \lambda_1 + \lambda_2) \pm \sqrt{(k_{21} + \lambda_1 + \lambda_2)^2 - 4\lambda_1\lambda_2} \right] . \quad (13)$$

The choice between these two theoretical solutions for k_{el} [and k_{12}, according to Eq. (11)] depends on additional experimental data, e.g., from the experiments with inhalatory application of the compounds (for butadiene, Fig. 2).

It should be mentioned that our analysis of exhalation curves after IP dosage of the volatile compound implies an "immediate" distribution within the organism (Cp_2). This holds true in so far as this process is much more rapid than those of exhalation and metabolism: just a few seconds after injection the compound is detectable in the gas phase; a "lag phase" is not observed. Moreover, the concentration curves in the gas phase are fully described by a biexponential function. For these reasons, a more complicate model is not needed in this case.

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