

Percutaneous uptake rate of 2-butoxyethanol in the guinea pig

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JOHANSON G, FERNSTRÖM P. Percutaneous uptake rate of 2-butoxyethanol in the guinea pig. *Scand J Work Environ Health* 12 (1986) 499–503. The percutaneous absorption rate and elimination kinetics of 2-butoxyethanol (ethylene glycol monobutyl ether) were estimated in the guinea pig. An intravenous bolus dose of 42 or 92 $\mu\text{mol/kg}$ of body weight was administered into the jugular vein of 10 pentobarbital-anesthetized animals. Epicutaneous administration of 2-butoxyethanol followed 2.5 h later in one or two sealed glass rings on the clipped back of the animal. Arterial blood samples were obtained and then analyzed for 2-butoxyethanol by gas chromatography. Following the intravenous dose, the apparent total clearance and mean residence time of 2-butoxyethanol were calculated to be $128 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (SD 30 %) and 4.7 min (SD 30 %), respectively. During the latter part of the 2-h skin exposure, the concentration of 2-butoxyethanol in the blood appeared to level off at an average concentration of $21 \mu\text{mol/l}$ (SD 45 %). The absorption rate through the skin was estimated to be 0.25 (range 0.05–0.46) $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ (SD 49 %). The skin uptake rate in the guinea pig was extrapolated to man for a comparison of the percutaneous absorption of liquid solvent with respiratory uptake of solvent vapor. The extrapolation indicated a risk of acute adverse effects when large areas of the skin are exposed to 2-butoxyethanol.

Key terms: blood, cellosolve, gas chromatography, glycol ether, toxicokinetics.

Glycol ethers are commonly used in consumer products, as well as in industrial processes, because of their excellent solvent and evaporation characteristics (19). Among the glycol ethers, the 2-alkoxyethanols (ethylene glycol monoalkyl ethers), and more specifically 2-ethoxyethanol (along with its acetate) and 2-butoxyethanol (ethylene glycol monobutyl ether), are the most common (13). Recent interest has focused on the reproductive hazards of some of the glycol ethers (9, 10). In general these solvents have rather low vapor pressures, compared to those of many other organic solvents, but they may readily penetrate the skin. Such penetration has been shown indirectly from the recording of various toxic effects, eg, the median lethal dose, after skin application of several glycol ethers (10). Two cases of transcutaneous 2-methoxyethanol (ethylene glycol monomethyl ether) poisoning have been reported (16). However, quantitative data on skin uptake rates in vivo are scarce. The aim of the present investigation was to estimate quantitatively the percutaneous uptake rate of 2-butoxyethanol in the guinea pig.

Materials and methods

All chemicals were of analytical grade and purchased from Merck (Darmstadt, Federal Republic of Germany) unless otherwise stated. They were used without further

purification. The animals used were female outbred guinea pigs (JA Sahlin, Malmö, Sweden), supplied with standard pelleted feed for guinea pig and rabbit breeding (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum. Their body weights ranged from 517 to 760 g. (See table 1 in the Results and Discussion section.)

The animals were anesthetized with pentobarbital (60 mg/ml mebumalnatium, ACO, Solna, Sweden) intraperitoneally with a dose of 0.6 mg/kg of body weight and then kept under anesthesia throughout the experiment. A polyethylene catheter (PE50) was implanted in the left jugular vein. This catheter was used for the bolus administration of 2-butoxyethanol. A similar catheter was inserted into the right carotid artery for blood sampling. Flushing the catheters with 130 IU (0.5 ml) of heparin (Vitrum, Solna, Sweden) in the beginning of the experiment prevented clotting. After an intravenous bolus dose (approximately 42 or 92 $\mu\text{mol/kg}$ of body weight) of 2-butoxyethanol (10.7 mg/ml 2-butoxyethanol in 0.3 M sodium chloride), arterial blood was sampled after 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min. Thereafter the hair on the back of the animal was clipped, and one or two glass rings with an exposure area of 3.14 cm^2 each were glued with cyanoacrylate (cyanolit 201, Benneter, Stockholm, Sweden) to the skin, as previously described (11). After the glue had dried, a new "blank" blood sample was taken. At 150 min after the intravenous administration, each ring was filled with 1 ml of undiluted 2-butoxyethanol and sealed with a cover glass. Arterial blood was sampled at the same time intervals as after the intravenous dose. On each sampling occasion, two arterial blood samples of $100 \mu\text{l}$ were collected in capillary tubes. Heptanol (99 %, Sigma, St Louis, Montana) was added to the samples

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as an internal standard. Toluene extracts of the samples were derivatized with pentafluorobenzoyl chloride (Pierce, Beijerland, Holland) and analyzed by gas chromatography with electron capture detection as described elsewhere (12).

At steady state, the zero order of the intravenous (iv) infusion rate of a substance may be obtained as the product of its blood concentration ($C_{ss,b}$) and its total blood clearance (CL_b). If skin metabolism is negligible, the rate (R) of substance flow through the skin into the systemic circulation is equal to the intravenous infusion rate. Accordingly, the percutaneous uptake rate of 2-butoxyethanol was calculated as

$$R_{\text{skin}} = C_{ss,b} \times CL_b$$

in the present study. The average blood concentration after 90–120 min of skin exposure was assigned to $C_{ss,b}$. The clearance was calculated as:

$$CL_b = \text{dose}_{iv} / \int_0^\infty C_b dt = \text{dose}_{iv} / \text{AUC}_{iv(0-\infty)}$$

The area under the blood concentration time curve following the intravenous dose ($\text{AUC}_{iv(0-\infty)}$) was obtained by the trapezoidal method (17). The quotient of the blood concentration at 60 min and the elimination rate constant was added as a residual term. The elimination rate constant was calculated as the average slope of a log-linear plot of concentration versus time 60–120 min after the intravenous administration in all experiments (see figure 2 in the Results and Discussion section.)

Another approach to obtain the uptake rate is based on mass-balance. The amount (a) of substance which has penetrated into the body at any given time (t) may be expressed as the sum of the amount of solvent present in the body and that eliminated:

$$a_{\text{uptake}} = a_{\text{body}} + a_{\text{el}}$$

The amount of substance in the body at time t may be expressed as:

$$a_{\text{body}} = C_b \times V_{ss}$$

where C_b is the measured concentration in blood and V_{ss} is the apparent steady-state volume of distribution. The V_{ss} of 2-butoxyethanol was assumed to be 56 % of the body weight. This was the average value obtained in previously performed experiments in which men were exposed to 20 ppm of 2-butoxyethanol (12). The amount of substance eliminated at time t may be expressed as:

$$a_{\text{el}} = CL_b \times \int_0^t C_b dt = CL_b \times \text{AUC}_{\text{skin}(0-t)}$$

The clearance value was obtained as has already been described, and the area under the blood concentration time curve ($\text{AUC}_{\text{skin}(0-t)}$) was calculated by the trapezoidal method. Combining the equations yields:

$$a_{\text{uptake}} = C_b \times V_{ss} + CL_b \times \text{AUC}_{\text{skin}(0-t)}$$

The “invasion curve,” ie, the plot of a_{body} versus time, approached a straight line during the latter half of the skin exposure. (See figure 3 in the Results and Discussion section). The percutaneous uptake rate (R_{skin}) was obtained as the slope of the line fitted by linear regression to the values on a_{body} after 75–120 min of skin exposure. The time lag of the skin penetration was determined as the intercept between the regression line and the x-axis.

Results and discussion

The concentration of 2-butoxyethanol in blood declined rapidly following an intravenous bolus dose (figures 1 and 2). Estimates of total clearance and mean residence time for the 10 individual experiments are listed in table 1. The average clearance value of $128 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ of body weight corresponds to $2.7 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of liver based on a liver weight of 47.2 g/kg of body weight in the guinea pig (1). The corresponding value for man has been shown to be $0.8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of liver (12), while the intrinsic clearance in perfused rat liver was $2.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ (14). The relatively high clearance observed in the present study may be partly caused by pentobarbital (15). However, as long as the clearance of 2-butoxyethanol remains unchanged during an experiment, any interaction from pentobarbital will not affect the calculation of the skin uptake rate.

After the epicutaneous administration of 2-butoxyethanol, the concentration in blood rose rapidly and then appeared to approach a plateau level during the latter half of the exposure period (figure 1). The apparent plateau level averaged 21 (range 8–36) $\mu\text{mol/l}$. Accordingly, the uptake plots in figure 3 approach straight lines. The average uptake rate was estimated to be 0.25 (range 0.05–0.46) $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, and the two ways of calculating give almost identical results (table 1). The percutaneous uptake rate of 2-butoxyethanol in the present study is comparable to that obtained in vitro with human skin [$0.03 \text{ SD } 0.10$] $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ (7)] once the species differences are taken into consideration (2). Similar results were obtained with the analogues propoxyethyl acetate and ethoxyethyl acetate in vivo (0.12 – $0.18 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ and 0.11 – $0.22 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, respectively) and in vitro (0.16 and $0.28 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, respectively) in beagle dogs (8). The skin uptake rates of other polar solvents such as n-butanol [$0.12 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ in dogs (4)] and methyl n-butyl ketone [0.05 and $0.08 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ in two men (5)] are also of similar magnitude.

The time lag of 21–60 min observed in the present study is in accordance with that reported for the penetration of 2-butoxyethanol through human skin in vitro (7). The tissue binding of 2-butoxyethanol appears to be low in that the olive oil:water and the

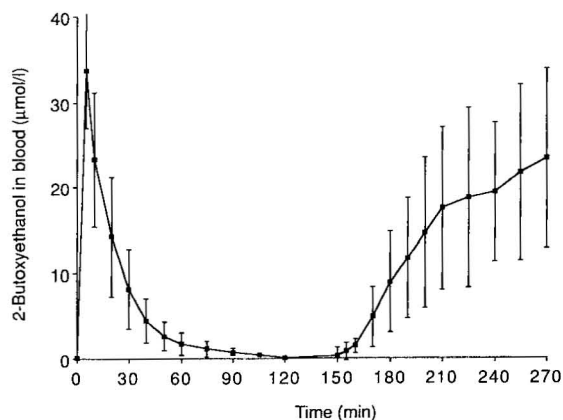


Figure 1. Time course of the average concentration of 2-butoxyethanol in the blood of nine guinea pigs given an intravenous dose (approximately 92 µmol/kg of body weight) at 0 min, followed by epicutaneous administration (area 6.28 cm²) of the solvent at 150 min. The bars represent the standard deviations.

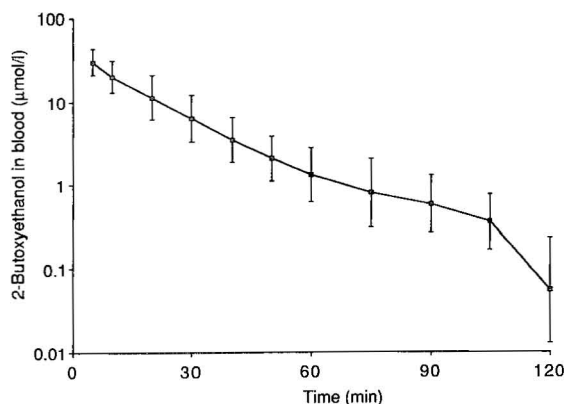


Figure 2. Time course of the concentration (geometric mean) of 2-butoxyethanol in the blood of nine guinea pigs given an intravenous dose (approximately 92 µmol/kg of body weight). The bars represent the geometric standard deviations. Note the logarithmic scale of the y-axis.

Table 1. Estimates of elimination kinetics and skin uptake of 2-butoxyethanol in the anesthetized guinea pig.

Experiment number	Body weight (kg)	Intravenous dose			Epicutaneous administration				
		Dose (µmol/kg)	CL _b ^a (ml · min ⁻¹ · kg ⁻¹)	t _b ^b (min)	Area (cm ²)	C _{ss,b} ^c (µmol/l)	R _{skin} ^d (nmol · min ⁻¹ · cm ⁻²)	R _{skin} ^e (nmol · min ⁻¹ · cm ⁻²)	Time lag ^f (min)
1	0.760	42	86	6.5	3.14	4	93	93	21
2	0.571	91	91	6.1	6.28	29	244	247	26
3	0.597	91	106	5.3	6.28	12	119	116	36
4	0.625	92	130	4.3	6.28	36	466	462	32
5	0.600	94	133	4.2	6.28	16	200	195	60
6	0.635	92	187	3.0	6.28	17	330	318	36
7	0.560	92	75	7.4	6.28	8	52	52	40
8	0.539	89	111	5.0	6.28	32	303	301	31
9	0.567	91	182	3.1	6.28	20	329	327	34
10	0.517	92	140	4.0	6.28	20	235	230	35
Mean ^g	0.579	91.5	128	4.7		21.2	253	250	36.8
SD (%) ^g	7	1	30	30		45	49	49	26

^a Total blood clearance, calculated as $CL_b = \text{dose}/AUC$, where AUC is the area under the blood concentration time curve of 2-butoxyethanol following a known intravenous dose.

^b Mean residence time, calculated as $\bar{t} = V_{ss}/CL_b$, on the assumption that the steady-state volume of distribution (V_{ss}) is 56 % of the body weight, as in man (12).

^c Steady-state concentration in blood, calculated as the average concentration at 90, 105, and 120 min.

^d Skin uptake rate, calculated as $R_{skin} = C_{ss,b} \times CL_b$.

^e Skin uptake rate, obtained as the slope of the straight line fitted to the "invasion curve" (figure 3).

^f Time lag of the skin penetration, obtained as the intercept with the x-axis of the straight line fitted to the "invasion curve" (figure 3).

^g Experiment 1 was excluded, as a different dose and exposure area were used.

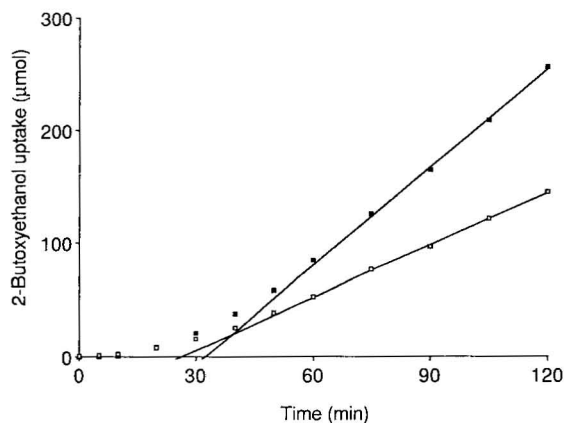


Figure 3. Estimated systemic uptake ("invasion curve") of 2-butoxyethanol in guinea pigs following epicutaneous administration of the solvent. While all the curves had similar shapes, only those from experiments 2 and 4 are presented.

bovine erythrocytes: water partition ratios are close to or less than unity (Johanson, unpublished observations) and in that the volume of distribution for 2-butoxyethanol is of the same order of magnitude as that of total body water (12). These pieces of information support the assumption that a constant absorption rate was virtually reached within the 120 min of skin exposure in the present study.

One may suspect that pentobarbital, used as the anesthetic in the present experiments, caused altered blood flow through the skin. It is however generally accepted that the stratum corneum is the main diffusive barrier (2, 18) and therefore that, with the exception of very small and diffusible substances, such as inert gases, the absorption rate is not affected by the blood flow.

The calculations presented in this study are valid only when the kinetics of 2-butoxyethanol are linear, or first order. In experiments performed with perfused rat liver, the elimination kinetics of 2-butoxyethanol were Michaelis-Menten like, the estimated values on the apparent Michaelis constant ranging from 0.32 to 0.70 mmol/l (14). The blood concentrations of 2-butoxyethanol were approximately 10 to 20 times lower in the present study. Linear kinetics are thus indicated under the present conditions, when extrapolating from rat to guinea pig.

It is of interest to relate the obtained results to the pulmonary uptake rate in man and to the occupational exposure limit. The absolute and relative respiratory uptake in men exposed to 2-butoxyethanol at 20 ppm (the current Swedish exposure limit) during light physical exercise was approximately 10 μ mol/min and 60 %, respectively (12). The corresponding area of skin exposure to the liquid solvent would be approximately 40 cm² if it is assumed that the absorption rate through human skin equals the average value obtained in the guinea pig in the present study. This assumption is conservative in that it probably overestimates the absorption rate in man (2). Conversely, dipping both hands in 2-butoxyethanol [area 740 cm² (5)] would result in an uptake rate of 185 μ mol/min, which corresponds to a theoretical inhalation exposure at 370 ppm during light physical exercise. At rest, and on the assumption of a respiratory ventilation of 6 l/min and a relative uptake of 60 %, the corresponding exposure concentration would be above the saturation level of 1 000 ppm. For comparison, the 4-h median lethal concentration is 450–486 ppm for rats (6) and the corresponding 7-h value for mice is 700 ppm (20). Humans experimentally exposed to 98 ppm, 113 ppm, and 195 ppm of 2-butoxyethanol vapor have been shown to experience discomfort and, in some cases, increased erythrocyte osmotic fragility (3).

In conclusion, skin contact with chemical products containing 2-butoxyethanol should be considered in industrial hygiene. Furthermore, exposure of large areas of the skin to 2-butoxyethanol may cause acute adverse effects in man.

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References

- Breazile JE, Brown EM. Anatomy. In: Wagner JE, Manning PJ, ed. *The biology of the guinea pig*. Academic Press, New York, NY 1976, pp 53–62.
- Bronaugh RL, Maibach HI. In vitro models for human percutaneous absorption. In: Maibach HI, Lowe NJ, ed. *Models in dermatology*. Volume 2. Karger, Basel 1985, pp 178–188.
- Carpenter CP, Pozzani UC, Weil CS, Nair JH, Keck GA, Smyth HF. The toxicity of butyl cellosolve solvent. *Arch Ind Health* 14 (1956) 114–131.
- Di Vincenzo GD, Hamilton ML. Fate of n-butanol in rats after oral administration and its uptake by dogs after inhalation or skin application. *Toxicol Appl Pharmacol* 48 (1979) 317–325.
- DiVincenzo GD, Hamilton ML, Kaplan CJ, Krasavage WJ, O'Donoghue JL. Studies on the respiratory uptake and excretion and the skin absorption of methyl n-butyl ketone in humans and dogs. *Toxicol Appl Pharmacol* 44 (1978) 593–604.
- Dodd DE, Snellings WM, Maronpot RR, Ballantyne B. Ethylene glycol monobutyl ether: Acute, 9-day and 90-day vapour inhalation studies in Fischer 344 rats. *Toxicol Appl Pharmacol* 68 (1983) 405–414.
- Dugard PH, Walker M, Mawdsley SJ, Scott RC. Absorption of some glycol ethers through human skin in vitro. *Environ Health Perspect* 57 (1984) 193–197.
- Guest D, Hamilton ML, Deisinger PJ, DiVincenzo GD. Pulmonary and percutaneous absorption of 2-propoxyethyl acetate and 2-ethoxyethyl acetate in beagle dogs. *Environ Health Perspect* 57 (1984) 177–184.
- Hardin BD. Reproductive toxicity of the glycol ethers. *Toxicology* 27 (1983) 91–102.
- Illing HPA, Tinkler JJB. Toxicity review 10: Glycol ethers. Health and Safety Executive, London 1985. (ISBN 0 11 883807 5).
- Jakobson I, Wahlberg JE, Holmberg B, Johansson G. Uptake via the blood and elimination of 10 organic solvents following epicutaneous exposure of anesthetized guinea pigs. *Toxicol Appl Pharmacol* 63 (1982) 181–187.
- Johanson G, Kronborg H, Näslund PH, Byfält Nordqvist M. Toxicokinetics of inhaled 2-butoxyethanol (ethylene glycol monobutyl ether) in man. *Scand J Work Environ Health* (in press).
- Johanson G, Rick U. Förekomst av glykoletrar i kemiska produkter i Sverige [Occurrence of glycol ethers in chemical products in Sweden]. Arbetskyddsverket, Stockholm 1986. (Arbete och hälsa 1986: 13). (English abstract).
- Johanson G, Wallén M, Byfält Nordqvist M. Elimination kinetics of 2-butoxyethanol in the perfused rat liver — Dose dependence and effect of ethanol. *Toxicol Appl Pharmacol* 83 (1986) 315–320.
- Nies AS, Shand DG, Wilkinson GR. Altered hepatic blood flow and drug disposition. *Clin Pharmacokinet* 1 (1976) 135–155.
- Ohi G, Wegman DH. Transcutaneous ethylene glycol monomethyl ether poisoning in the work setting. *J Occup Med* 20 (1978) 675–676.
- Rowland M, Tozer TN. *Clinical pharmacokinetics: Concepts and applications*. Lea & Febiger, Philadelphia, PA 1980.
- Scheuplein RJ. Permeability of the skin: A review of

- major concepts and some new developments. *J Invest Dermatol* 67 (1976) 672—676.
19. Smith RL. Review of glycol ether and glycol ether ester solvents used in the coating industry. *Environ Health Perspect* 57 (1984) 1—4.
 20. Werner HW, Nawrocki CA, Mitchel JL, Miller JW, von Oettingen WF. Effects of repeated exposures of rats to

vapors of monoalkyl ethylene glycol ethers. *J Ind Hyg Toxicol* 25 (1943) 374—379.

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