Chapter 2: Section 2.1

Percutaneous absorption of neat and aqueous solutions of 2-butoxyethanol in volunteers
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Abstract

Objectives: To study the influence of the presence of water on the dermal absorption of 2-butoxyethanol (BE) in volunteers.

Methods: Six male volunteers were dermally exposed to 50%, 90% or neat w/w BE for 4 h on the volar forearm over an area of 40 cm². An inhalation exposure with a known input rate and duration served as a reference dosage. The dermal absorption parameters were calculated from 24-h excretion of total (free + conjugated) butoxyacetic acid (BAA) in urine and BE in blood, measured after both inhalation and dermal exposure.

Results: The dermal absorption of BE from aqueous solutions was markedly higher than that of neat BE. The time-weighted average dermal fluxes were calculated from the urine and blood data and expressed in milligrammes per square centimetre per hour. The dermal fluxes obtained from cumulative 24-h excretion of BAA amounted to 1.34 ± 0.49, 0.92 ± 0.60 and 0.26 ± 0.17 mg cm⁻² h⁻¹ for 50%, 90% and neat BE, respectively. The dermal fluxes calculated from the BE blood data amounted to 0.92 ± 0.34 and 0.74 ± 0.25 mg cm⁻² h⁻¹ for 50% and 90% BE, respectively. The permeation rates into the blood reached a plateau between 60 and 120 min after the start of exposure, indicating achievement of steadystate permeation. The apparent permeability coefficient Kp, was 1.75 ± 0.53·10⁻³ and 0.88 ± 0.42·10⁻³ cm h⁻¹ for 50% and 90% BE, respectively.

Conclusion: The percutaneous absorption of BE from aqueous solution increased markedly when compared with neat BE. Even water content as low as 10% led to an approximate fourfold increase in the permeation rates. These findings are important for the health risk assessment of occupational exposure to BE, since BE is commonly used in mixtures that contain water. Exposure to aqueous solutions of 50% and 90% of BE may result in substantial skin absorption: if a 60-min skin contact of 1000 cm² is assumed, dermal uptake would be fourtimes higher than the pulmonary uptake of an 8-h occupational exposure at a TLV of 100 mg m⁻³. This clearly justifies the skin notation for BE. For the purpose of biological monitoring, both BE in blood and BAA in urine were shown to be reliable indicators of exposure.
Introduction

Ethylene glycol ethers are frequently used in industry and households as solvents, emulsifiers and detergents. The use of 2 ethoxyethanol and 2-butoxyethanol (BE) has increased after the removal of 2-methoxyethanol from nearly all formulations because of its toxicity [19]. They are used in great quantities because of their excellent hydrophilic and lipophilic properties. Because of the low vapour pressure and high rate of dermal absorption, significant systemic exposure can occur through contact with the skin [5, 6]. It has been shown that BE readily penetrates the skin in guinea pigs and rats in vivo and in human, guinea pig and rat skin in vitro [1, 4, 5, 6]. The presence of water has been shown to enhance the percutaneous absorption of BE in vivo in guinea pig skin and in vitro in human skin [6, 20]. Percutaneous absorption of neat BE in humans has been demonstrated [7, 9]; however, dermal absorption from aqueous solutions of BE has not been studied. Since BE is commonly used in water mixtures, it is relevant to compare the absorption rate of neat BE and that of aqueous solutions in humans.

Material and methods

Subjects
Six male volunteers, aged 22–55 years and with no history of dermatological disease, participated in this study. They were in good health, had no visible skin damage and used no medication. The Ethical Committee of the Academic Medical Center, University of Amsterdam, approved the experiment protocol. Written informed consent was obtained from all subjects prior to experiments.

Reference inhalatory exposure
Each volunteer inhaled the solvent vapour for 30 min through a mouthpiece with a two-way valve connected to a Tedlar (DuPont, Delaware, USA) bag. The concentration of the vapour in the bag was approximately 93 ± 6.8 mg m⁻³ (mean value of six exposures), which is below the present occupational exposure limit in the Netherlands (100 mg m⁻³) [11]. In order to determine the respiratory input rate we measured the total exhaled volume.

Dermal exposure
A bottomless glass chamber (area 40 cm²) was placed on the volar forearm and filled with 8 ml of dosing BE solution. To prevent leakage, we glued the glass chamber onto the skin using UHU-Hart glue (UHU, Bul’hl, Germany). The concentration of BE
in the solution was measured before and after exposure. In order to avoid inhaling solvent vapour during the application of the solvent, the volunteer sat in a ventilated clean-air cabin with overpressure, and put his arm through an opening in the wall of the cabin. The exposure lasted for 4 h. Blood samples were collected for 8 h (16 samples per experiment). Urine samples were collected every 4 h during the 24-hour period. Each volunteer was exposed twice to a 50% BE solution (exposure on two different arms), once to 90% and once to neat BE. The period between two dermal exposures of the same skin site was at least 4 weeks.

Analytical methods

Chemicals
Acetone (p. a.), dichloromethane (p. a.), n-hexane (Lichrosolv), hydrochloric acid (conc., 37%), methanol (Lichrosolv), potassium carbonate (p. a.) and pyridine were purchased from Merck (the Netherlands). Phenoxethanol (98%) and ethoxyacetic acid (98%) were purchased from Aldrich (the Netherlands). Pentafluorobenzoyl chloride (99%) and pentafluorobenzyl bromide (≥ 99%) were purchased from Fluka (the Netherlands). Butoxyethanol (99%) was purchased from Sigma (the Netherlands) and butoxyacetic acid from TCI (Japan).

Analysis of BE in plasma
Immediately after blood collection in Li-heparin tubes, the plasma samples were prepared and stored in safe-lock tubes at 18 °C until required for analysis. BE in plasma was determined with a slightly modified method of Johanson and Fernstrom [5]; and Johanson et al. [8], which is based on extraction with dichloromethane and derivatization with pentafluorobenzoyl chloride and electron capture detection (ECD). The limit of quantitation (LOQ) of the method was 0.014 mg L⁻¹ and the coefficient of variation was 7%. Gas chromatographic (GC) analysis was carried out with a Hewlett-Packard 5890 GC (Hewlett-Packard, USA) equipped with a63Ni ECD. Two AT-1701 capillary columns (30 m:0.25 mm, 0.25-μm film thickness; Alltech, The Netherlands) were connected by glass connector. The initial column temperature was 50 °C, and the temperature was increased to 240 °C at 35 °C min⁻¹ and held for 16 min. The injector temperature was 250 °C, the detector temperature was 260 °C and the column head pressure was 150 kPa. The sample (1 μL) was injected by means of the splitless injection technique.
Analysis of BAA in urine

After collection, 1.5 ml aliquots of urine were stored in safe-lock tubes at -18 °C until required for analysis. For 50% BE, the concentration of BAA was determined in all collected samples. Since the excretion of BAA was shown to be completed within a 24-h period, the concentration of BAA after exposure to 90% and neat BE was determined only in pooled 24-h urine. The analysis of BAA in urine samples was based on acid hydrolysis of conjugated BAA, subsequent derivatization with pentafluorobenzyl bromide (PFBBr) and GC-ECD analysis. For that purpose 50 µL of concentrated HCl was added to 50 µL of urine and heated for 60 min at 95 ± 5 °C. After the solution had cooled to room temperature, 2 ml of acetone, ± 0.15 g of potassium carbonate, 20 µL of ethoxyacetic acid solution (100 mg L⁻¹) as an internal standard and 20 µL of PFBBr were added and heated for 60 min at 95 °C. After being cooled to room temperature, a 100- µL aliquot of the acetone layer was transferred to a safe-lock tube containing 250 µL of 90% methanol in water and 750 µL of n-hexane. Samples were vortexed for 5 min and centrifuged for 30 s (11,860 g). GC analysis was carried out with a Carlo Erba HRGC 5300 GC (Interscience, The Netherlands) equipped with a 63Ni ECD. The column was HP-1 (25 m-0.32 mm, 0.52- µm film thickness, Alltech, The Netherlands). The initial column temperature was 100 °C, and the temperature was increased to 170 °C at 5 °C min⁻¹ and subsequently to 200 °C at 45 °C min⁻¹ and held for 1 min. The injector and detector temperatures were 250 °C and the column head pressure was 100 kPa. The sample (3 µL) was injected by the split injection technique (split ratio 1:50). The LOQ of the method was 3.3 mg L⁻¹ and the coefficient of variation was 14%.

Calculations

Inhalation exposure

The respiratory input rate (IR) was calculated as follows [12]:

\[
IR (\mu g \text{ min}^{-1}) = C_{inh} \times (V_{inh} / t_{exp} - f \times V_d)
\]

where \(V_{inh}\) (L) is total inhaled volume, \(C_{inh}\) (µg L⁻¹) is the concentration in inhaled air, \(t_{exp}\) (min) is duration of exposure, \(V_d\) (L) is dead-space volume taken as sum of the anatomical dead space (0.15 L) and the dead space of the mouthpiece (0.04 L) and \(f\) (vent min⁻¹) is individual ventilation frequency. We calculated the amount absorbed after inhalation exposure (INHabs) by multiplying IR by exposure duration (30 min).
Dermal exposure

The amount of BE absorbed into the skin (DER_{abs}) was calculated from the excreted BAA measured after both inhalation (BAA_{inh}) and dermal (BAA_{der}) exposure as follows:

\[ \text{DER}_{abs} \text{ (mg)} = \frac{\text{BAA}_{der}}{\text{BAA}_{inh}} \times \text{INH}_{abs} \]

We calculated the average dermal flux throughout the exposure by dividing the amount of BE absorbed into the skin by exposure area and exposure time and expressed it in milligrammes per square centimetre per hour.

For the calculation of permeation rates and dermal fluxes we used the linear system dynamics method that is extensively described elsewhere [12, 13]. Briefly, we determined individual systemic kinetics from the reference inhalation experiment using the blood BE concentration-time data. Using a convolution method we fitted the data to a mathematical expression combining the kinetic response after bolus dose with exposure duration and concentration. The parameters obtained from a fitted function and the concentration-time data after dermal exposure were used to determine the permeation rates as function of time by deconvolution. The total amount of BE absorbed into the blood was determined from the area under the permeation rate-time curve. The amount of BE absorbed into the skin during exposure was considered to be equal to the amount absorbed into the blood. We calculated the average dermal flux from blood data in the same manner as from urine data, by dividing the amount absorbed into the skin by exposure area and exposure time, and expressed it in milligrammes per square centimetre per hour. The maximum permeation rates were determined from the slope of the cumulative absorbed mass vs time. When steady-state permeation is achieved the maximum permeation rate represents the apparent permeability coefficient K_p (cm h^{-1}).

The principle of the method is illustrated in Fig 1, which shows the concentration-time course in blood following (a) inhalation, (b) dermal exposure and (c) corresponding permeation rate-time courses of BE.

Using the results of the (two) replicated dermal exposures, we calculated the intra-subject variability as well as the inter-subject variability in a restricted sense, i.e. after eliminating the intra-subject variability. For the latter we used the coefficient of variation = \{([\text{between subject variance}] / \text{within subject variance}) / 2\}^{1/2}/\text{mean}.
Fig 1a-c: The linear system dynamics method used for the estimation of permeation rates and dermal fluxes in one subject exposed to 50% BE. (a) Concentration-time course of BE after inhalation exposure and the fitted function; (b) concentration-time course after dermal exposure; (c) estimated permeation rate-time course. Inhalation exposure concentration was 93 mg m$^{-3}$ for 30 min; dermal exposure area was 40 cm$^2$ and dermal exposure duration was 4 h.

Results

The amount of BE in dosing solutions measured before and after dermal exposure to 50 % BE were 49.5 % and 49.0 ± 1.4 % (mean of 12 individual exposures), respectively. This indicates that the concentration throughout the exposure was constant (i.e. the dose was infinite). The concentration of BE in blood after exposure to neat BE was, in most of the samples, below the detection limit of the method. After exposures to 50 % and 90 % BE, the BE concentrations could be measured in all subjects at all time points. In none of the volunteers did skin irritation occur; however, after exposure the skin had a wrinkled appearance.
Figure 2 shows the typical concentration–time courses of BE in blood, measured after exposure to 50%, 90% and neat BE in one volunteer. In all subjects, exposure to 50% and 90% BE resulted in higher blood concentrations than those after exposure to neat BE.

This is consistent with the higher 24-h cumulative excretion of BAA after exposure to aqueous solutions of BE than that after exposure to neat BE (Fig 3). As calculated from the individual BAA concentration-time curves determined after inhalation exposure, the average half-life of BAA amounted to 3.4 h (range 1.3 to 3.8 h). This implies that BAA is almost completely excreted in urine within 24 h of the start of the exposure. Using the amount of BE absorbed after inhalation exposure (the average value for six subjects was 20.9 ± 5.0 mg) and the cumulative excretion of BAA after inhalation, we calculated that, on average, 57% (range 42–70%) of absorbed BE was excreted as BAA.
Fig 3: Cumulative excretion of BAA in urine in a subject dermally exposed to 50%, 90% and neat BE. For neat BE only cumulative amount was measured.

Table 1. Average dermal fluxes of BE after exposure to 50%, 90% and neat BE obtained from blood and urine data. Values are means ± SD. ND not determined: blood concentrations were below limit of quantitation.

<table>
<thead>
<tr>
<th>Average dermal flux (mg cm⁻² h⁻¹)</th>
<th>50 % BE</th>
<th>90 % BE</th>
<th>Neat BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on blood data</td>
<td>0.92 ± 0.34 (n=6)</td>
<td>0.74 ± 0.25 (n=3)</td>
<td>² ND (n=3)</td>
</tr>
<tr>
<td>Based on urine data</td>
<td>1.34 ± 0.49 (n=6)</td>
<td>0.92 ± 0.60 (n=5)</td>
<td>0.26 ± 0.17 (n=6)</td>
</tr>
</tbody>
</table>

¹ Each subject was exposed twice to 50 % BE
² Not determined: blood concentrations below limit of quantitation
Chapter 2: Section 2.1

The average dermal fluxes calculated from the BAA urine data as well as from the BE blood data are presented in Table 1. For the exposures to 50% and 90% BE the time courses of permeation rates were also determined. The permeation rates reached a plateau between 60 and 120 min after the start of exposure, indicating steady-state permeation. The maximum permeation rates, which are in fact the apparent permeability coefficients, are presented in Table 2.

Table 2. Apparent permeability coefficients of 50%, 90% and neat BE obtained from blood data. Values are means ± SD. ND not determined: blood concentrations were below limit of quantitation.

<table>
<thead>
<tr>
<th>Apparent permeability coefficients (x 10⁻³ cm h⁻¹)</th>
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<tbody>
<tr>
<td>50 % BE</td>
</tr>
<tr>
<td>1.75 ± 0.53</td>
</tr>
<tr>
<td>(n =6)</td>
</tr>
</tbody>
</table>

¹Each subject was exposed twice to 50 % BE
²Not determined: blood concentrations below limit of quantitation

In order to obtain insight into intra-individual and inter-individual variation of dermal fluxes, we performed dermal exposure to 50% BE twice for each volunteer. The intra-individual and inter-individual variance of the dermal fluxes obtained from the blood and urine data are shown in Table 3. Table 3 shows that the inter-individual variation was approximately twice as high as the intra-individual variation, regardless of whether urine or blood data were taken.

Table 3. Intra-individual and inter-individual variations of dermal fluxes of BE after exposure to 50% BE

<table>
<thead>
<tr>
<th>Mean ± SD (mg cm⁻² h⁻¹)</th>
<th>90 % Confidence interval</th>
<th>Intraindividual CV (%)</th>
<th>Interindividual CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on blood data</td>
<td>0.92 ± 0.34</td>
<td>0.63 – 1.19</td>
<td>16</td>
</tr>
<tr>
<td>Based on urine data</td>
<td>1.34 ± 0.49</td>
<td>0.94 – 1.74</td>
<td>20</td>
</tr>
</tbody>
</table>
In order to assess the relevance of our results for occupational exposure to BE, we estimated the contribution of dermal uptake to the total uptake. The uptake after dermal exposure of 1000 cm$^2$ for 1 h was compared with an 8-h inhalatory uptake at TLV in the Netherlands [11]. Table 4 shows that such dermal exposure to aqueous solutions under the above-described exposure conditions leads to a substantial uptake that even exceeds the pulmonary uptake.

Table 4. Estimated dermal uptake of BE in relation to the pulmonary uptake during an 8-h exposure at the TLV (100 mg m$^{-3}$)

<table>
<thead>
<tr>
<th>Pulmonary uptake (mg)</th>
<th>Dermal uptake (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 % BE</td>
</tr>
<tr>
<td>346</td>
<td>1340</td>
</tr>
</tbody>
</table>

$^1$Assumed minute volume of 10 L/min and alveolar retention of 72 % [9]
$^2$Assumed dermal exposure of 1000 cm$^2$ skin for 1 hour

Discussion and conclusions

The presented results show that the presence of water in the applied solution markedly increases the percutaneous absorption of BE in humans in vivo. The time-weighted average fluxes calculated from the 24-h cumulative urine BAA excretion amounted to 0.26, 0.92 and 1.34 mg cm$^{-2}$ h$^{-1}$, for neat, 90% and 50% BE, respectively. The presence of water has previously been shown to enhance the percutaneous penetration of BE. In an in vivo study [6], the permeability coefficients (Kp) of BE in the guinea pig were consistent with those reported in our study and were $0.30 \times 10^{-3}$, $0.84 \times 10^{-3}$ and $1.82 \times 10^{-3}$ cm h$^{-1}$ for neat, 80% and 40% BE, respectively. The values of Kp that we obtained from blood results were $1.75 \pm 0.53 \times 10^{-3}$ and $0.88 \pm 0.42 \times 10^{-3}$ cm h$^{-1}$ for 50% and 90% BE, respectively. Wilkinson and Williams reported in an in vitro study with human skin considerably higher apparent Kp values with aqueous solutions than with undiluted doses [20]. They suggested that those findings could be explained by better partitioning of BE between the stratum corneum (SC) and vehicle (aqueous solution), resulting in a proportionately higher concentration gradient across the skin, which hence promoted steady-state flux. It has been shown for a number of hydrophilic compounds, e.g. for BE, propoxyacetic acid and alcohols, that hydration of the SC leads to enhanced absorption [6, 10, 16]. It seems to us unlikely that the changed composition of either vehicle or SC would influence the partition of BE between those two phases to such
an extent (almost a fourfold increase of dermal flux in the presence of 10% water). A possible explanation could be that water affects the structure of the SC, in particular its lamellar organization, leading to a higher porosity and improved permeation of BE [18]. Recently Tang et al. [17] have found that hydration leads to induction of new pores/reduction of the tortuosity of existing pores in the SC, improving the percutaneous penetration of water-soluble compounds. Another explanation of the promoting effect of water on the permeation of BE was argued by Corley et al., who suggested that BE may have “fixed” the skin at the higher concentrations [2]. However, the mechanism and the meaning of the “fixing” of the skin remained unclear in that study. It may also be that the use of neat BE will dehydrate the skin to a certain extent and that it will block the polar pathways through the skin.

Initially, we intended to use only blood BE concentrations to determine the dermal absorption kinetics. However, the absorption of neat BE was too low, and the blood BE concentrations were under the LOQ of the method used. In order to enable comparison of dermal fluxes of different BE concentrations, we also measured the urinary excretion of the main metabolite, BAA. Therefore, the comparison of dermal fluxes for all BE concentrations mainly relies on the BAA measured in urine, though dermal fluxes based on blood data of 50% and 90% BE were also calculated. The excretion of BAA in urine is of particular significance for occupational exposure and is used as a biological indicator of exposure [14, 15]. In most of the studies, only the free urinary fraction of BAA was measured. However, recent studies by Corley et al. [3], Rettenmeier et al. [14] and Sakai et al. [15] have shown that a substantial amount of BAA is excreted in human urine as a glutamate conjugate (on average 48, 71 and 66%, respectively), and that the conjugated BAA fraction is highly variable. This was the reason why, in the present study, we measured the excretion of the total (i.e. free and conjugated) BAA using the acid hydrolysis procedure proposed by Rettenmeier et al. [14]. The average half-life of BAA in the present study was 3.4 h (range 1.3 to 3.8 h), which was in good agreement with values reported by Corley et al. (mean value 3.3 h, range 2.4 - 4.4 h) [3]. This half-life implies that the excretion of BAA was almost completed by 24 h.

The 24-h cumulative amount of BAA excreted in urine measured after inhalation accounted for 57% of the respiratory uptake of BE (range 42 to 70%). This was higher than the recovery of 17–55% reported in an inhalatory volunteer study by Johanson et al.; however, in that study only excretion of free BAA was measured [8].
Chapter 2: Section 2.1

The measurement of only non-conjugated BAA might also explain high intra-individual and inter-individual variation in cumulative excretion of BAA after dermal exposure of volunteers to neat BE. The absorption rates reported in that study varied highly, from 0.05 to 0.74 cm h⁻¹, suggesting that analysis of BAA might not be a reliable method for the assessment of the dermal absorption of BE. With regard to the variability of the absorption rates, the biological variability plus analytical variability in the flux through the skin of an individual was 16% (blood) and 20% (urine), assuming the systemic kinetics constant. If the inhalation exposure had also been replicated, a somewhat higher value would have been found (and a lower value for the inter-individual variation). These data are relevant in studies where subjects are compared on the basis of their skin permeability.

A comparison of the dermal uptake of aqueous solutions of BE with the respiratory uptake at the current occupational exposure limit for BE revealed substantial skin absorption. If one assumes a 60-min skin contact of 1000 cm² with a 50% or 90% aqueous solution of BE, the uptake would be three to four times higher than the pulmonary uptake of the 8-h occupational exposure.

In conclusion, dermal uptake of BE increases markedly in the presence of water. This has already been reported in vivo in guinea pigs and in in vitro studies with human skin; however, this is the first time that it has been shown in humans in vivo. These findings should therefore be considered in the health risk assessment of occupational dermal exposure to BE where water-based products containing glycol ethers are used.

BE showed substantial dermal absorption: if we assume 60-min skin contacts of approximate areas of 1000 cm² with 50% or 90% BE, dermal uptake would exceed the pulmonary uptake of the 8-h TLV exposure. This clearly justifies the skin notation for BE, and indicates biological monitoring (BM) as the preferred approach to exposure assessment. For the purpose of BM, both BE in blood or BAA in urine can be used. When BAA is chosen, total BAA instead of only free BAA in urine should be determined.
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References


