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Absorption of Some Glycol Ethers Through Human Skin *in Vitro*

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To assist evaluation of the hazards of skin contact with selected undiluted glycol ethers, their absorption across isolated human abdominal epidermis was measured *in vitro*. Epidermal membranes were set up in glass diffusion cells and, following an initial determination of permeability to tritiated water, excess undiluted glycol ether was applied to the outer surface for 8 hr. The appearance of glycol ether in an aqueous "receptor" phase bathing the underside of the epidermis was quantified by a gas chromatographic technique. A final determination of tritiated water permeability was compared with initial values to establish any irreversible alterations in epidermal barrier function induced by contact with the glycol ethers. 2-methoxyethanol (EM) was most readily absorbed (mean steady rate 2.82 mg/cm^2/hr), and a relatively high absorption rate (1.17 mg/cm^2/hr) was also apparent for 1-methoxypropan-2-ol (PM). There was a trend of reducing absorption rate with increasing molecular weight or reducing volatility for monoethylene glycol ethers (EM, 2.82 mg/cm^2/hr; 2-ethoxyethanol, EE, 0.796 mg/cm^2/hr; 2-butoxyethanol, EB, 0.198 mg/cm^2/hr) and also within the diethylene glycol series: 2-(2-methoxyethoxy) ethanol (DM, 0.206 mg/cm^2/hr); 2-(2-ethoxyethoxy) ethanol (DE, 0.125 mg/cm^2/hr) and 2-(2-butoxyethoxy) ethanol (DB, 0.035 mg/cm^2/hr). The rate of absorption of 2-ethoxyethyl acetate (EEAc) was similar to that of the parent alcohol, EE. Absorption rates of diethylene glycol ethers were slower than their corresponding monoethylene glycol equivalents. Combination of intrinsic toxicity and ability to pass across skin contribute to assessment of hazards of contact with undiluted glycol ethers.

The importance of the percutaneous route for absorption of solvents and other chemicals encountered in the workplace is now recognized. Assessment of the hazards of skin contact with potentially toxic materials requires a combination of estimates of uptake via the skin and quantitative toxicology. Diffusion across the nonliving outer layer of the skin, the stratum corneum, is the rate-limiting step for most situations of percutaneous absorption (*1*). Because the permeability properties of the stratum corneum are unchanged by removal from the body, *in vitro* experiments are appropriate and offer a number of advantages over whole animal or human volunteer experiments (*1,2*). The large differences in permeability which exist between human skin and that of certain laboratory animals (*3–5*) make human skin the preferred tissue for assessments of percutaneous absorption. Accordingly, the absorption of certain glycol ethers from undiluted liquids was measured through human abdominal epidermis *in vitro* and the physical effects of contact with the glycol ethers on stratum corneum assessed by "before and after" tritiated water permeability measurements.

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Material and Methods

Materials

Glycol ethers employed in this study are listed in Table 1 and the abbreviations of compound names are also shown. The glycol ethers were supplied by ICI Petrochemicals and Plastics Division (Wilton UK) and were of greater than 98% purity, with the exception of PM which contained approximately 95% 1-methoxypropan-2-ol.

Tritiated water was obtained from the Radiochemical Centre (Amersham, UK) and diluted with distilled water to 5 μCi/mL.

The liquid scintillation medium used was FisoFluor (Fisons PLC, Loughborough UK).

Analytical Techniques

Gas chromatographic techniques employing flame ionization detection were used for the quantification of glycol ethers. The instruments used were either a Pye Unicam (Cambridge UK) Model GCD or a Hewlett Packard Model HP 5730 fitted with 150 cm, 2 mm ID glass columns packed with Tenax GC (monoglycol

Table 1. Glycol ethers used and general details.

Compound	Synonym	Abbreviation	Molecular weight	Boiling point, °C	GC column temperature, °C
2-Methoxyethanol	Ethylene glycol monomethyl ether	EM	76	124	150
2-Ethoxyethanol	Ethylene glycol monoethyl ether	EE	90	135	140
2-Ethoxyethyl acetate	Ethylene glycol monoethyl ether acetate	EEAc	132	156	180
2-*n*-Butoxyethanol	Ethylene glycol monobutyl ether	EB	118	171	180
1-Methoxypropan-2-ol	Propylene glycol monomethyl ether	PM	90	120	145
2-(2-Methoxyethoxy) ethanol	Diethylene glycol monoethyl ether	DM	120	194	190
2-(2-Ethoxyethoxy) ethanol	Diethylene glycol monomethyl ether	DE	134	197	190
2-(2-*n*-Butoxyethoxy) ethanol	Diethylene glycol monobutyl ether	DB	162	230	215

series) or Tenax TA (diethylene glycol series). Nitrogen (30 mL/min) was the carrier gas. GC column temperatures appropriate for each glycol ether are shown in Table 1. All GC samples were 2 μL. A range of standard solutions in distilled water was prepared for each glycol ether and a standard curve of GC response versus concentration drawn for each glycol ether. Appropriate standard samples were chromatographed at frequent intervals during analysis.

For radiochemical assay, 25-L samples were taken with Hamilton microsyringes and added to FisoFluor (10 mL) in plastic vials. Radioactivity was assayed in an Intertechnique SL30 scintillation spectrometer. Samples gave a constant counting efficiency in the media employed and therefore no quench correction was necessary.

Preparation of Epidermal Membranes

Human abdominal whole skin (dermis plus epidermis) was obtained post mortem. The subcutaneous fat was removed and the skin immersed in water at 60°C for 45 sec. The epidermis was then gently separated from the dermis and the epidermal sheet was then floated on water and picked up onto aluminum foil.

Skin Absorption Measurements

Epidermal membrane discs, 3 cm in diameter, were cut with a punch, floated on water and drawn over the "receptor" chamber of diffusion cells of either of the types shown in Figure 1. The experimental conditions were identical whether diffusion cells having horizontal or vertical membrane orientation were used. The donor chambers of the diffusion cells were clamped in place to give 1.8 cm^2 of epidermal surface available for absorption. All receptor solutions were stirred by a Teflon-coated stirring bar and experiments were conducted at 30°C.

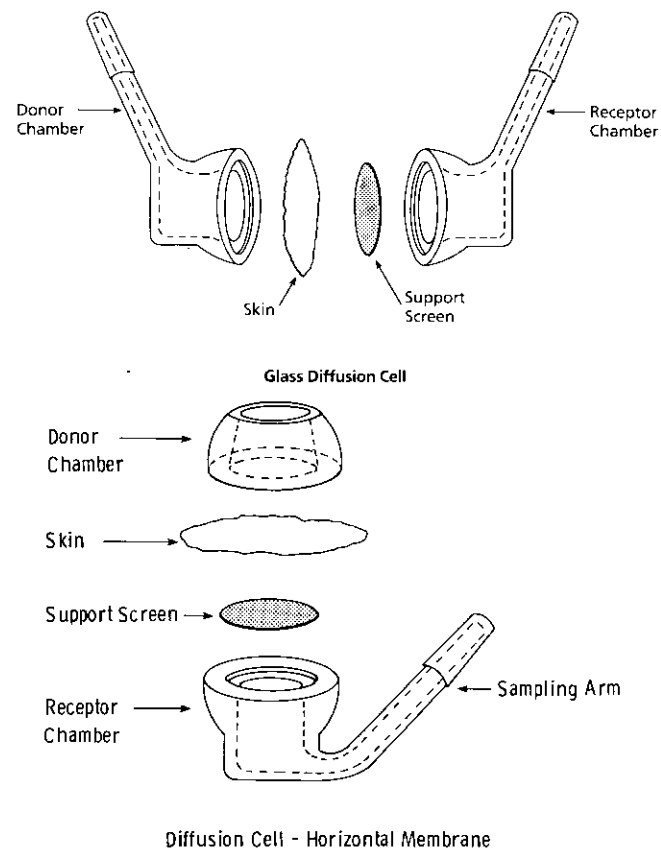

FIGURE 1. Glass diffusion cells.

The integrity of epidermal membranes was established before and after contact with the glycol ethers by measurement of their permeability to tritiated water. The donor chambers of diffusion cells were filled with tritiated water (5 μCi/mL), and the appearance of radiolabel in a measured volume of distilled water in receptor chamber was followed with time. Samples (25 μL) were taken hourly from the third to the sixth hour

after addition of the donor tritiated water. Donor solutions were sampled at zero and 6 hr. A permeability constant for tritiated water was calculated from the slope of the (linear) plot of "sample counts versus times" as follows:

$$\text{Permeability constant (cm/hr)} = \frac{\text{Slope (cpm/25 } \mu\text{L/hr)} \times \text{Receptor volume (mL)}}{\text{Mean donor count (cpm/25 } \mu\text{L)} \times \text{Area of skin (cm}^2\text{)}}$$

Epidermal membranes displaying tritiated water permeability constants greater than 1.5×10^{-3} cm/hr in the initial determination were deemed to have been damaged in preparation and were rejected. Tritiated water was desorbed from acceptable membranes into distilled water in donor and receptor membranes overnight and both chambers were emptied prior to measurement of glycol ether absorption.

For assessment of glycol ether absorption, the donor chamber of each diffusion cell (vertical membrane) was filled (circa 5 mL) with undiluted glycol ether or 1 mL placed in the donor of diffusion cells with horizontal membranes. Receptor chambers were filled with a known volume of distilled water. In each experiment a "control" diffusion cell was employed in which the donor chamber was left empty but distilled water placed in the receptor and sampled so that interfering compounds could be recognized. Samples were taken hourly from the receptors (0.25 mL, monoglycol series) or half-hourly (0.75 mL, diethylene glycol compounds) and glycol ether content assayed in duplicate 2 μL aliquots of the receptor samples as described above. Each receptor sample was replaced with an equal volume of fresh distilled water. All glycol ether experiments were run for 8 hr. The results of the assay of receptor solution glycol ether content were converted to total amount having penetrated (with correction for sample removal) and plotted against time. The slope of this plot gives the rate of absorption of glycol ether through 1.8 cm^2 of epidermis, simple division yielding rate per 1 cm^2. Permeability constants for the glycol ethers were calculated from the linear, steady-state, region of the above plots by dividing the rate per unit area by the applied concentration.

After 8 hr contact, the donor glycol ethers and receptor solutions were removed and replaced with distilled water overnight. The distilled water wash was removed and the second determination of membrane permeability to tritiated water made. The ratio of final tritiated water permeability constant to the initial value, the "damage ratio," provides an indication of irreversible alterations in epidermal diffusion barrier function occurring during the experiment.

Results

The absorption of all glycol ethers achieved maximum steady rates within the experimental period (Figs. 2 and 3). The mean steady rates and the permeability constants derived from them are shown in Table 2. EM was the most readily absorbed glycol ether of the series tested. PM, EE, and EEAc were also readily absorbed, while EB was the most slowly absorbed monoglycol ether. EE and its acetate exhibited similar rates of absorption. For the three diethylene glycol ethers, DM, DE, and DB, rates of absorption were significantly slower than their monoethylene glycol equivalents, EM, EE and EB, respectively.

The time course of absorption of the diethylene glycol ethers is illustrated in Figure 2, where plots of total amount penetrated versus time are shown (mean values for all samples at each time point). The lag time is determined by extrapolating the linear portion of these curves to zero absorption. This parameter is used to compare the early time course of absorption for different compounds (*6*) but tends to be difficult to determine with accuracy. However, the lag time displayed by DB

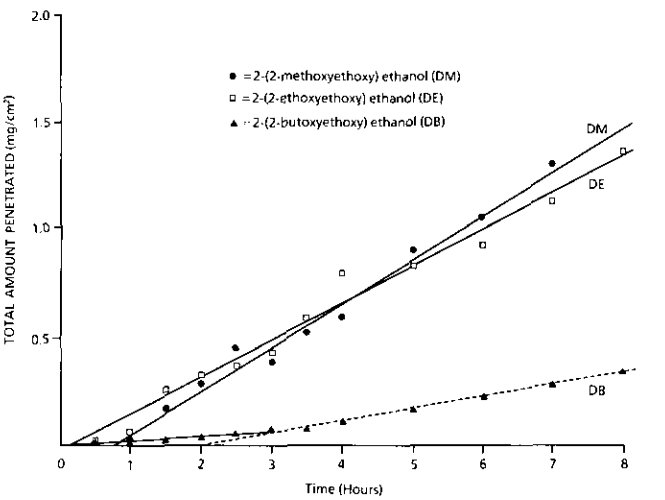

FIGURE 2. Time-course of diethylene glycol ethers absorption through human epidermis.

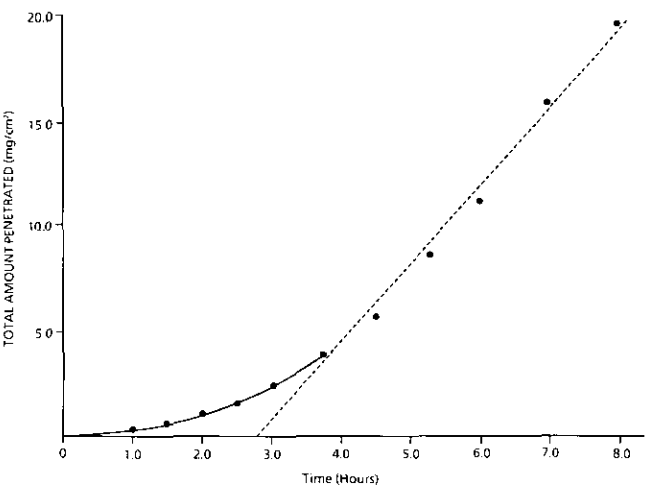

FIGURE 3. Time-course of 2-methoxyethanol (EM) absorption through human epidermis.

Table 2. Skin absorption of glycol ethers.

Compound	Permeability constant, cm/hr × 10^4	Rate of absorption, mg/cm²/hr[a]	Damage ratio[a]	Approx. lag time, hr
2-Methoxyethanol (EM)	28.9	2.82 ± 2.63 (22)	3.51 ± 1.47 (20)	1–3
2-Ethoxyethanol (EE)	8.42	0.796 ± 0.460 (11)	2.74 ± 1.46 (10)	<1
2-Ethoxyethyl acetate (EEAc)	8.07	0.800 ± 0.430 (10)	1.75 ± 1.08 (10)	< 1
2-Butoxyethanol (EB)	2.14	0.198 ± 0.700 (8)	2.07 ± 0.88 (8)	< 1
1-Methoxypropan-2-ol (PM)	12.5	1.170 ± 1.070 (11)	3.38 ± 1.82 (10)	< 1
2-(2-Ethoxyethoxy) ethanol (DM)	2.06	0.206 ± 0.156 (11)	3.16 ± 1.77 (10)	1
2-(2-Ethoxyethoxy) ethanol (DE)	1.32	0.125 ± 0.103 (10)	1.20 ± 2.62 (8)	< 1
2-(2-Butoxyethoxy) ethanol (DB)	0.357	0.035 ± 0.025 (9)	2.05 ± 1.01 (5)	2

[a]Results are mean ± standard deviation with number of determinations in parentheses.

(Fig. 2 and Table 2) of approximately 2 hr was clearly longer than for other glycol ethers (Table 2) with the exception of EM. The apparent lag time for EM (Fig. 3) varied between 1 and 3 hr, and this is discussed below.

Control damage ratio values for water contact alone lie between 1.0 and 2.0. Thus slight irreversible effects on barrier function occurred for EM, EE, PM and DM.

Discussion

The results of the skin permeability measurements for individual glycol ethers show high degrees of variability (standard deviations, Table 2); however, a range of permeability properties is expected in any human population. The conditions of contact between the glycol ethers and the skin are rigorous in the diffusion cell system and thus represent a "worst case" situation of prolonged contact with excess solvent.

Within the monoglycol series of glycol ethers, there is a trend of reducing absorption rates with increasing molecular weight or reducing volatility. A similar trend is apparent for the diethylene glycol compounds. The diethylene glycol liquids are less rapidly absorbed through human epidermis than the corresponding monoethylene glycol members and this may be related to the additional polar ether group rather than simply an increase in molecular size.

The lag times displayed by the glycol ethers in this study were generally less than 1 hr and the longer period required for DB (lag time 2 hr) is compatible with the slow absorption rate for this liquid. Ideally, the lag time relates to the period required to achieve a full, linear concentration gradient of penetrant across the thickness of barrier stratum corneum. For EM, the extended period of increasing absorption rate may be reversible due to solvent-induced alterations in permeability.

Table 3. Effects of solvent contact (6–8 hr) on the permeability of human epidermis to water.

Liquid	Damage ratio[a]
Toluene	5.5
Aniline	2.3
Chlorobenzene	13
2-Phenyl ethanol	2.3
Benzylamine	> 1000
Water	1–2

[a]Damage ratio is the ratio of skin permeability to tritiated water after contact with a test compound to that recorded before such contact.

Some slight degree of irreversible damage may also have been induced by EM, EE, PM, DM (compare Table 2 and 3) but this is unlikely to have had a significant effect on permeability. It cannot be judged from experiments reported here whether the glycol ethers altered permeability in a reversible manner while in contact with the epidermis.

In comparison with absorption rates for other undiluted liquids (Table 4) which have been measured for human epidermis using similar techniques, it may be seen that EM, and PM achieve relatively high rates. The rapid absorption of EM through human skin is in agreement with the clinical observations and conclusions of Ohi and Wegman (*8*) and the *in vivo* human measurements of blood levels following skin contact with EM made by Nakaaki et al. (*9*). The results of this study and those of Nakaaki et al. (*9*) cannot be compared quantitatively because the relationship between blood levels and absorption is not known. Other absorption rates for glycol ethers span a wide range of observed rates for other liquids (compare Tables 2 and 4).

When considering the hazards of skin contact with undiluted glycol ethers, rates of absorption alone are insufficient information. Three types of data must be considered: absorption, nature of probable skin contact and the intrinsic toxic potential of the chemical

Table 4. Absorption through skin from undiluted liquids.

Liquid	Absorption rate, mg/cm^2/hr
Di-*n*-butyl phthalate	0.0066[c]
Dimethyl phthalate	0.033[c]
n-Butanol	0.06[a]
Benzyl alcohol	0.073[c]
2-Phenyl ethanol	0.26[c]
Ethanol	0.57[a]
Methanol	8.4[a,b]

[a]Data of Scheuplein and Blank (7).
[b]Methanol causes extensive damage to stratum corneum.
[c]Dugard, P. H. (unpublished data).

concerned. In general, the results of dermal toxicity tests are not easy to use in quantitative assessments of human hazard because of the unknown relationship between animal and human skin permeability and the uncertainties of the dosing procedures employed. However, skin contact usually leads to an extended period of absorption akin to slow intravenous infusion. There is no "first-pass" metabolism in the liver, as may occur in oral intake, although the capacity of the skin to metabolize materials during absorption is unknown. The toxicity data which indicate "potency" and which may be most easily related to numerical estimates of uptake through skin are those from inhalation toxicology studies. Thus, the rate of uptake during inhalation leading to a known effect may be compared with the rates of absorption during specific examples of skin contact.

For the glycol ethers tested, it is apparent that the high toxicity of EM combined with high permeability of the skin leads to concern regarding the hazards of skin contact with this material. Apparently less toxic, slowly absorbed materials, such as EB, are of lower concern for similar examples of skin contact.

The results of this study employing undiluted glycol ethers should not be extrapolated directly to solvent mixtures on a "rate proportional to concentration" basis, because components of the mixture may have a variety of effects on the absorption process. Therefore, it is recommended that assessments of absorption are made when skin contact with a glycol ether-containing mixture is expected.

The Chemical Manufacturers Association (USA) commissioned the studies on diethylene glycol ethers.

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