Matthew J Traynor[i]

Simon C Wilkinson[i]

Elanor Ball[ii]

Faith M Williams[i]

The influence of water mixtures on the dermal absorption of glycol ethers

i) Toxicology Unit, Medical School and Institute for Research on Environment and Sustainability, University of Newcastle, Newcastle Upon Tyne, NE1 7RU, UK.

ii) Health and Safety Executive, Magdalen House, Stanley Precinct, Bootle, Merseyside, L20 3QZ, UK.

Fax +44 (0)191 222 6442

Email : M.J.Traynor@newcastle.ac.uk

Address for correspondence

Faith Williams

Toxicology Unit

Devonshire Building

University of Newcastle Upon Tyne

Newcastle Upon Tyne

NE1 7RU

Abstract

Glycol ethers are solvents widely used in industrial and household products. They have been shown to have a range of severe toxic effects in man following absorption and metabolism to their aldehyde and acid metabolites. This study assessed the influence of water mixtures on the dermal absorption of butoxyethanol and ethoxyethanol *in vitro* through human and rat skin. Studies were performed using dermatomed skin in Newcastle Dick flow through cells under occluded conditions. Butoxyethanol penetrated human skin up to 6 fold more rapidly from aqueous solution (50%, 450mg/ml) than from the neat solvent. Similarly penetration of ethoxyethanol was increased three fold in the presence of water (50%, 697 mg/ml). There was a corresponding increase in apparent permeability coefficient as the glycol ether concentration in water decreased. The maximum penetration rate of water also increased in the presence of both glycol ethers.

Key words

Butoxyethanol, ethoxyethanol, water mixture, *in vivo*, *in vitro*, skin absorption.

Introduction

Glycol ethers are widely used in industrial and household applications because their chemical and physical properties make them versatile solvents, miscible with both water and organic media. Glycol ethers penetrate the skin rapidly (Kezic et al, 1997; Filon et al 1999) and it has recently been reported that following a one hour exposure of the human forearm to a 50% butoxyethanol 50% water mixture body levels exceeded the 8 hour threshold limit value for respiratory exposure to butoxyethanol (Jakasa et al, 2004). The toxicity of glycol ethers to man is mainly caused by the aldehyde and acid metabolites (Ghanayem et al, 1987) after conversion by alcohol dehydrogenase and aldehyde dehydrogenase. (Hepatic metabolism plays a major role in the fate of topically applied compounds (Aasmoe *et al,* 1998). Skin has been shown to contain alcohol and aldehyde dehydrogenases (Kao and Carver 1990; Hewitt et al, 2000) but local metabolism of glycol ethers during dermal penetration has not been demonstrated (Lockley et al, 2002; 2004), suggesting that the rapid passage of the solvents through the skin limits access to the enzymes.

Butoxyethanol has been demonstrated to cause erythrocyte haemolysis (Dartsch et al, 1999) following metabolism to butoxyacetic acid (Bartnik et al, 1987; Ghanayem et al 1989). Ethoxyethanol has been shown to have haematological, (Aasmoe et al, 1998) developmental effects and reproductive effects (Hardin 1983; Hardin et al, 1984) in laboratory animals. There is also some evidence for human effects in exposed workers (Welch and Cullen 1988). Due to the ease with which the glycol ethers are absorbed through the skin (Johanson et al, 1988; Kezic et al 1997; Filon et al, 1999) and the potential for development of adverse health effects it is important to understand factors that influence absorption. There have been a number of studies of dermal

absorption of glycol ether vapours in humans *in vivo* (Johanson and Boman 1991; Kezic et al, 1997) and of undiluted liquids (Johanson et al, 1988; Kezic et al, 1997). Jakasa *et al* 2004 also investigated the absorption of aqueous solutions of glycol ethers in man compared to studies with human skin *in vitro*. The ability of *in vitro* studies to predict *in vivo* absorption of neat butoxyethanol and ethoxyethanol and butoxyethanol or ethoxyethanol in methanol in the rat has been demonstrated (Lockley *et al* 2002, 2004). The aim of this study is to use *in vitro* methods to determine factors that influence the absorption of ethoxyethanol and butoxyethanol water mixtures through human and rat skin compared to a polydimethylsiloxane membrane.

Materials and methods

^{14}C 2-butoxyethanol, specific activity 54mCi/mmol was obtained from Amersham. Tritiated water specific activity 1600 MBq/ml was purchased from ICN radiochemicals (Basingstoke, UK) and ^{14}C 2-ethoxyethanol, specific activity 2.08 μCi/mg was a gift from Unilever. Minimum essential medium (Eagle) and gentamycin were obtained from Sigma, sodium hydrogen carbonate and teepol, were purchased from BDH, Hisafe 3 scintillation fluid was obtained from Fisher. 2-butoxyethanol was obtained from Fluka and 2-ethoxyethanol from Aldrich. The water used was sterile water for irrigation. The polydimethylsiloxane membrane which was used in a parallel study of absorption of methyl paraben (Chilcott *et al* 2005) was donated by Dr R Chilcott DSTL (Product code 19TO.3-1000-60M1 SAMCO Silicone Products)

Skin Preparation

28 day old male Wistar rats were sacrificed by cervical dislocation, the dorsal and abdominal regions were shaved and the skin dissected. The skin was placed dermal side down on a corkboard and dermatomed to a thickness of 280μm.

Human breast skin was obtained after cosmetic surgery from a local hospital and stored at -70ºC until required. Ethical approval for obtaining skin was given by the University of Newcastle Medical and Dental Ethics Committee and the University Hospital of South Durham Ethics.

A section of skin was removed from the freezer and allowed to defrost. The skin was then placed dermal side down on a corkboard and dermatomed to a thickness of 320µm. All experiments were performed using human skin from at least two donors.

A synthetic (polydimethylsiloxane) membrane (thickness 400 ± 13 µm) was used in some experiments to compare absorption between human and rat skin and a synthetic alternative. The membrane was prepared for use by soaking it in sterile water for 24 hours prior to use.

In vitro flow through diffusion system.

The system consisted of 15 teflon flow through cells of the Scott Dick-Newcastle design. Receptor fluid (Eagles minimum essential medium containing 2.2g/L sodium hydrogen carbonate and 200µg/ml gentamycin, pH 7.4 maintained by gassing with CO_2/air) was pumped through the cells at 1.5ml/h using a peristaltic pump. The receptor fluid reservoir and cells were maintained at 32ºC using a water jacket connected to a circulating water bath.

Skin sections were placed in the diffusion cells (exposed surface area 0.64cm^2) and secured in place using threaded nuts. The cell was partially occluded by a tight fitting cap containing carbon filters.

Dose application and determination of diffusion.

Butoxyethanol was used neat (900mg/ml) and at 810mg/ml, 675mg/ml, 450mg/ml, 90mg/ml, 45mg/ml, 9mg/ml, 4.5mg/ml, and 0.9mg/ml in aqueous solution. Doses

were prepared by the addition of 2µl ^{14}C butoxyethanol per 1ml of final dose. Ethoxyethanol was used neat (930mg/ml) and at 837mg/ml, 697mg/ml, 465mg/ml, 93mg/ml, 46.5mg/ml, 9.3mg/ml, 4.65mg/ml, and 0.93mg/ml in aqueous solution. Doses were prepared by the addition of 2µl ^{14}C ethoxyethanol per 1ml of final dose. H_2O was applied neat and as a 1:1 (v/v) mixture with butoxyethanol and/or ethoxyethanol. 10µl $^{3}H_2O$ was added per 1ml of final dose solution.

Aliquots, 128µl (equivalent to 200µl/cm^2), of the dose were applied to the exposed surface of the skin at time zero. Receptor fluid fractions (0.75ml) were collected using a fraction collector every 30 minutes for the first three hours and then every 60 minutes (1.5ml) until the experiments were terminated at 20 hours. In human skin experiments, the dose was removed from the skin surface at 4 hours using alternate wet (3% teepol) and dry tissue swabs (6 swabs in total). For studies with rat skin and membrane the dose remained in contact with the skin for the full study and the dose remaining on the skin surface at termination of the study was recovered in the same way. Finite dose studies were also performed for butoxyethanol doses using 12.8 µl of solution (equivalent to 20µl/cm^2).

Determination of distribution.

Receptor fluid fractions were weighed for accurate determination of amount of fluid collected and 250µl aliquots were removed for scintillation counting. The tissue swabs used to remove the dose solution and the carbon filters from the traps above each cell were soaked in 10ml scintillation fluid mixed and left to stand for two hours before counting. At the end of the experiment the skin was removed from the cells and digested using 2ml of 1.5M potassium hydroxide in 4:1(v/v) methanol:water.

Once the skin was fully digested (72 hours), glacial acetic acid (70µl) was added to quench chemiluminesence and 10ml of scintillation fluid was added prior to counting.

Calculation of absorption parameters

Amounts of radioactivity in receptor fluid samples were used to construct a cumulative absorption-time curve for the various test compounds. Steady state flux (J) values were calculated from the slope of the linear region of cumulative absorption versus time graph. The applied dose was an infinite dose and the apparent permeability coefficient (K_p) was calculated by dividing the flux rate by the concentration of the test compound in the dose solution. The lag time was obtained from the intercept of the cumulative absorption time curve on the time axis. Absorption parameters were compared using one-way ANOVA followed by Post hoc testing using Bonferroni's correction.

Results

Absorption of butoxyethanol water mixtures through skin *in vitro*.

Rat skin was more permeable than human skin resulting in a maximum penetration rate for neat butoxyethanol of 0.39±0.06 mg/cm^2/h for human skin and 0.73±0.01 mg/cm^2/h for rat skin (Tables 1 and 5). The maximum penetration rate for butoxyethanol increased when butoxyethanol was diluted with water to a maximum of 2.34±0.28 mg/cm^2/h from a 450 mg/ml solution in human skin (Figure 1) and to a maximum of 1.46±0.09 mg/cm^2/h from a 810 mg/ml (Figure 2) solution for rat skin. The majority of the dose in the human skin experiments was removed from the skin surface unabsorbed after four hours (Figure 3). The apparent Kp increased as the applied concentration of glycol ether decreased for both human and rat skin but there was no change in time to steady state at any concentration in either species. There was evidence of a small reservoir effect in the skin butoxyethanol was detected in the receptor fluid for up to four hours after the dose was removed. Finite dose studies found a similar pattern of increased absorption of butoxyethanol from solutions containing water compared to neat butoxyethanol (Table 3).

The maximium absorption rate of neat butoxyethanol through the polydimethylsiloxane membrane was 9.05±.0.82 mg/cm^2/h and the apparent Kp was 10±0.9 x 10^{-3} cm/h (Table 4). In contrast to both human and rat skin the maximum penetration rate decreased as the dose concentration was decreased and the apparent Kp remained constant.

Absorption of ethoxyethanol water mixtures through skin *in vitro*.

The maximum penetration rate of neat ethoxyethanol was 0.64±0.09 mg/cm^2/h. This increased in the presence of water to a maximum of 1.87±0.31 mg/cm^2/h from a 697 mg/ml solution (Figures 4 and 5). The apparent Kp increased from 0.69±0.09 x10^{-3} cm/h for neat ethoxyethanol to 3.2±0.5 x10^{-3} cm/h from a 465mg/ml solution (Table 3). There was no significant change in time to steady state for any dose solution. As with butoxyethanol the majority of the applied dose was removed unabsorbed after four hours (Figure 6).

Absorption of water through skin

The maximum penetration rate of water through human skin was 4.72±0.52 mg/cm^2/h. This figure was slightly increased when water was mixed with butoxyethanol or ethoxyethanol, despite the proportional decrease in the concentration of the water (Table 6). The apparent Kp of water was also found to increase in the presence of one or both of the glycol ethers (Table 6). There was no significant change in time to steady state for any of the tested doses.

Discussion

All of these studies were performed under partially occluded conditions (cell covered with a charcoal trap which allowed air passage through the mesh) and the skin was exposed to an infinte dose (large volume, concentration not depleted during the study) test solutions for a period of four hours and then washed off. This mimics *in vivo* studies performed elsewhere (Jakasa, *et al*. 2004).

In the current study the highest maximum penetration rate for butoxyethanol through human skin was from a 450 mg/ml solution (50 % solution) (2.34±0.28 mg/cm^2/h) from a 450 mg/ml solution, a value more than six times greater than the maximum penetration rate of neat butoxyethanol (0.39±0.06 mg/cm^2/h) . Addition of only 10% water to the neat solvent resulted in the maximum penetration rate almost doubling, while addition of 90% water with associated dilution of the butoxyethanol dose resulted in a maximum penetration rate that was still greater than neat butoxyethanol. These results clearly indicated that water enhanced the absorption of butoxyethanol through human skin *in vitro.* The maximum penetration rate of butoxyethanol achieved from the finite doses was significantly lower than from the inifine dose as steady state absorption was never truly achieved. However the increase in butoxyethanol absorption from the finite doses was observed as for the infinite doses. This is significant as the finite dose is more relevant to occupational exposure scenarios where a small splash of spilt glycol ether may come in to contact with a workers exposed skin.

The results using rat skin showed a similar effect of water on the absorption of butoxyethanol although the proportional increase was not as great as that seen with human skin. The results obtained using the polydimethylsiloxane membrane were different to those with human or rat skin. The maximum penetration rate for the membrane decreased as the dose concentration decreased. The apparent permeability coefficient was independent of dose concentration in contrast to human and rat skin where the apparent Kp increased with decreased dose concentration.

The maximum penetration rate for neat ethoxyethanol through human skin in this study was 0.64±0.09 mg/cm^2/h which was in agreement with Filon *et al (1999)* who found a maximum penetration rate of 0.83±0.4 mg/cm^2/h for ethoxyethanol when testing the absorption of a range of glycol ethers. The greatest flux rate for ethoxyethanol was seen from a 50% aqueous solution, where a flux rate three fold greater than that for neat ethoxyethanol was observed. (table 3). As with butoxyethanol a substantial increase in flux rate was observed with addition of only 10% water to the dose solution. The apparent permeability coefficient of ethoxyethanol was also increased in the presence of water up to a maximum with a 50% aqueous solution (table 3). There was no change in lag time for any of the tested doses.

The dermal absorption of water also increased in the glycol ethers mixtures. When the amount of water present in the dose solution was halved there was a slight increase in the maximum penetration rate of water in the presence of both butoxyethanol and ethoxyethanol. The apparent permeability coefficient of the water also increased.

The mechanism of the increased penetration of glycol ethers from aqueous solution has not been fully explained. Several factors may contribute to the increase in maximum penetration rate and Kp in the presence of water . An increase in Kp suggests an alteration in the barrier properties of the stratum corneum. Changes in permeability result in increased and more rapid partitioning of the glycol ethers into the stratum corneum. It has been shown that neat solvents such as ethanol dehydrate the stratum corneum (Pillai *et al* 2004, Marjukka Suhonen *et al* 1999) When dehydrating solvents are applied to the skin mixed with water the disruptive effect on stratum corneum lipid structure and barrier function may increase the permeability to both the solvent and water (Van der Merwe and Riviere 2005). It has been suggested that neat butoxyethanol may also have a dehydrating effect on the skin contributing to less flux, so that with the addition of water an increase in absorption would occur (Jakasa *et al*, 2004). A further suggestion is that the water affects the structure of the stratum corneum leading to higher porosity and enhanced absorption of the butoxyethanol (Tezel *et al*, 2003).

The absolute absorption rate of water was greater than butoxyethanol and in butoxyethanol mixtures it might enhance butoxyethanol penetration by solvent drag. It is also known that at certain concentrations, butoxyethanol in water does not behave as a perfect solution (Castillo and Dominguez 1990). At these concentrations the butoxyethanol molecules cluster together in pseudomicelles and if these were in contact with skin might preferentially partition out of the water and into the lipid rich stratum corneum. According to Raoults' law if a solvent of lower vapour pressure (butoxyethanol 0.1 Kpa) is added to one of higher vapour pressure (water 2.34 Kpa) the amount of solvent evaporating decreases. Therefore in a butoxyethanol/water

mixture the butoxyethanol would decrease evaporation of the water thus keeping it in contact with the skin for longer, increasing the hydration of the skin and thus increasing the absorption of both the water and the butoxyethanol. However this would only occur if the dose is not truly infinite and if the lag time is very long, so is unlikely to have effected the infinite dose data from this study but may have contributed to the effect observed with finite doses.

One question that remains to be answered is whether the skin has been damaged following exposure to glycol ether/water mixtures and how this differs from the effects of water. Measurements of conductivity although variable between cells did not indicate major skin damage (results not included).

The unusual absorption profiles of the glycol ethers was previously reported for human skin *in vitro* by this group (Wilkinson and Williams 2002) and it has previously been reported that the presence of water increased the absorption of butoxyethanol through guinea pig skin *in vivo* (Johanson and Fernstrom 1988). Application of butoxyethanol and butoxyethanol/water mixtures to human volunteers *in vivo* under similar conditions to this study showed similar effects. (Jakasa, *et al*. 2004).

QSARs constructed with Kp values derived with saturated aqueous solutions allow derivation of Kp on the basis of log P and molecular weight for unknowns (Fitzpatrick *et al*, 2004) This approach is not applicable to a solvent which is freely soluble in water. For neat butoxyethanol absorption through rat skin was greater than human skin *in vitro*. However when water was added to the dose solution the effect was to

enhance the penetration of butoxyethanol greater with human than rat skin resulting in the rat data under predicting the absorption of aqueous solutions in humans.

In conclusion this study has found that the dermal absorption of the glycol ethers is increased in the presence of water. This is significant because the majority of industrial and household uses of glycol ethers are in aqueous form and systemic exposure may cause severe toxic effects.

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Table 1 Maximum penetration (flux) rates, apparent Kp and time to steady state for butoxyethanol applied at various concentrations in aqueous solution to dermatomed human skin. Figures are mean ± SEM (n ≥ 5). ** P<0.01 ***P<0.001 when compared to neat butoxyethanol.

Conc (mg/ml)	Flux (mg/cm^2/h)	Kp (x10^{-3} cm/h)	Lag Time (h)
900 (neat)	0.39±0.06	0.44±0.07	0.6±0.07
810 (90%)	0.72±0.11	0.88±0.14	0.6±0.04
675 (75%)	2.34±0.28***	3.41±0.41	0.6±0.02
450 (50%)	2.69±0.40***	5.95±0.89**	0.7±0.04
90 (10%)	0.66±0.14	7.26±1.52***	0.8±0.06
4.5 (5%)	0.22±0.01	4.73±0.28	0.8±0.05
9 (1%)	0.14±0.03	12.5±2.3***	0.5±0.04
4.5 (0.5%)	0.05±0.01	10.4±2.5***	0.7±0.07
0.9 (0.1%)	0.01±0.00	15.3±2.2***	0.5±0.09

Table 2 Maximum penetration (flux) rates, apparent Kp and time to steady state for finite doses containing butoxyethanol applied at various concentrations in aqueous solution to dermatomed human skin. Figures are mean ± SEM (n ≥ 5). ** P<0.01 when compared to neat butoxyethanol.

Conc (mg/ml)	Flux (mg/cm^2/h)	Kp (x10^{-3} cm/h)	Lag Time (h)
900 (neat)	0.04±0.01	0.49±0.12	1.4±0.6
810 (90%)	0.08±0.02	0.97±0.29	1.0±0.4
450 (50%)	0.19±0.03**	4.26±0.79**	0.8±0.4

Table 3 Maximum penetration (flux) rates, apparent Kp and time to steady state for ethoxyethanol applied at various concentrations in aqueous solution to dermatomed human skin. Figures are mean ± SEM (n ≥ 5). * P<0.05 ** P<0.01 ***P<0.001 when compared to neat ethoxyethanol

Conc (mg/ml)	Flux (mg/cm^2/h)	Kp (x10^{-3} cm/h)	Lag Time (h)
930 (neat)	0.64±0.09	0.69±0.09	0.9±0.05
837 (90%)	1.36±0.26	1.63±0.31	0.9±0.04
697 (75%)	1.87±0.31***	2.68±0.45**	1.1±0.03
465 (50%)	1.51±0.25*	3.28±0.54***	0.6±0.04
93 (10%)	0.05±0.00	0.57±0.22	1.0±0.01
46.5 (5%)	0.03±0.01	0.57±0.15	1.0±0.04
9.3 (1%)	0.004±0.00	0.5±0.08	0.9±0.06
4.65 (0.5%)	0.002±0.00	0.5±0.1	1.0±0.04
0.93 (0.1%)	0.004±0.00	0.5±0.06	0.9±0.05

Table 4 Maximum penetration (flux) rates, apparent Kp and time to steady state for butoxyethanol applied at various concentrations in aqueous solution to

polydimethylsiloxane membrane. Figures are mean ± SEM (n ≥ 5). ***P<0.001 when compared to neat butoxyethanol.

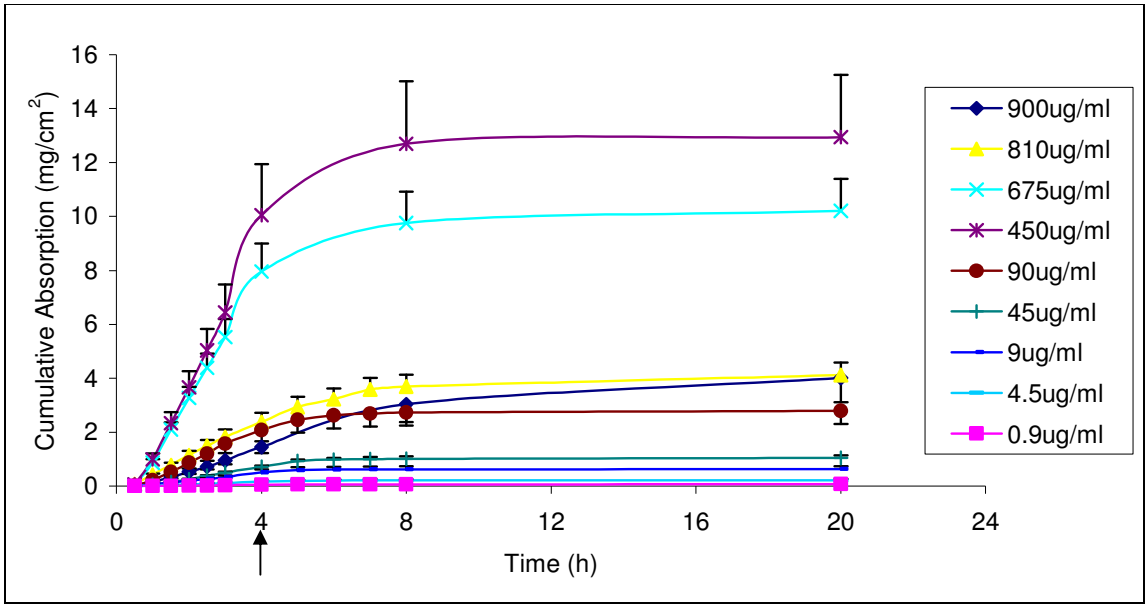
Conc (mg/ml)	Flux (mg/cm^2/h)	Kp (x10^{-3} cm/h)	Lag Time (h)
900 (neat)	9.05±0.82	10.06±0.91	0.4±0.05
810 (90%)	7.36±0.59	9.08±0.72	0.6±0.05
450 (50%)	4.89±0.22***	10.86±0.49	0.7±0.04

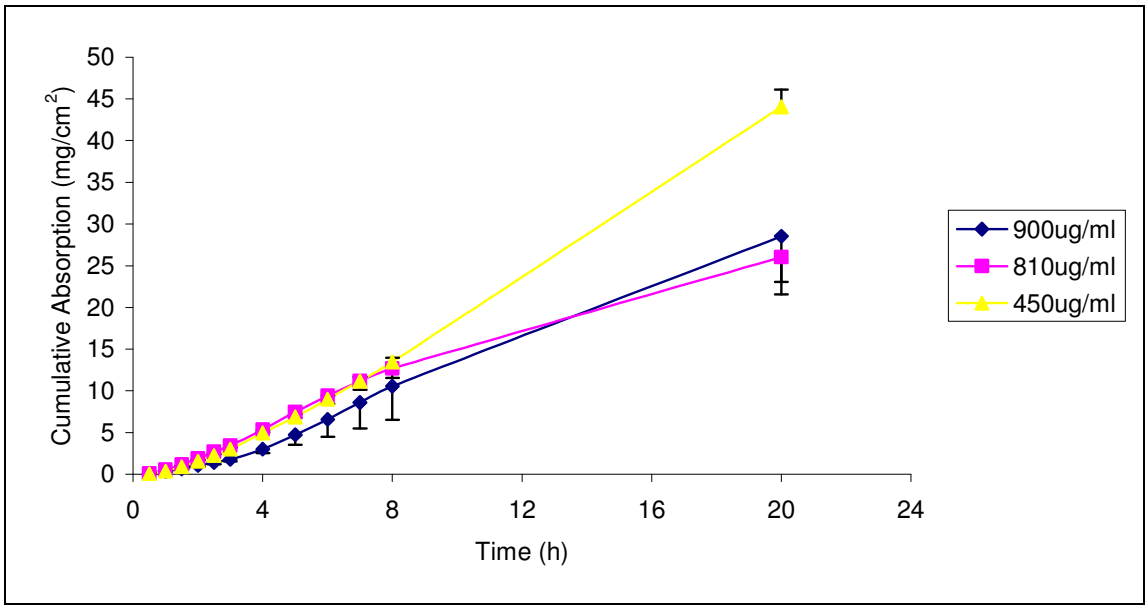
Table 5 Maximum penetration (flux) rates, apparent Kp and time to steady state for butoxyethanol applied at various concentrations in aqueous solution to dermatomed rat skin. Figures are mean ± SEM (n ≥ 5). *P< 0.05 ***P<0.001 when compared to neat butoxyethanol.

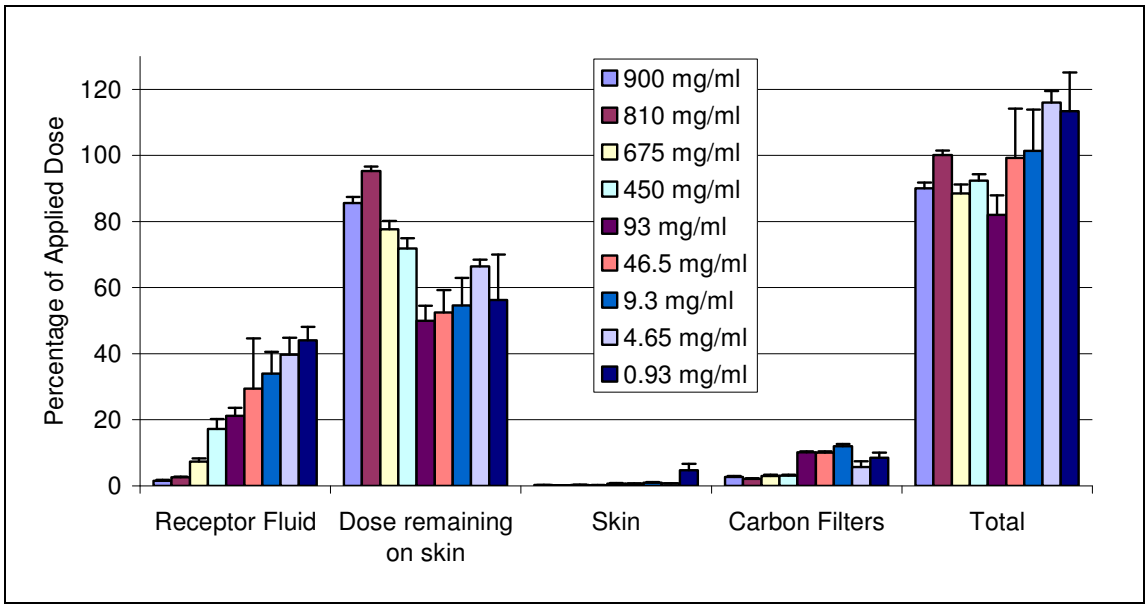
Conc (mg/ml)	Flux (mg/cm^2/h)	Kp (x10^{-3} cm/h)	Lag Time (h)
900 (neat)	0.73±0.11	0.81±0.12	0.6±0.06
810 (90%)	1.45±0.09	1.79±0.11*	0.7±0.05
450 (50%)	1.31±0.08	2.92±0.19***	0.8±0.07

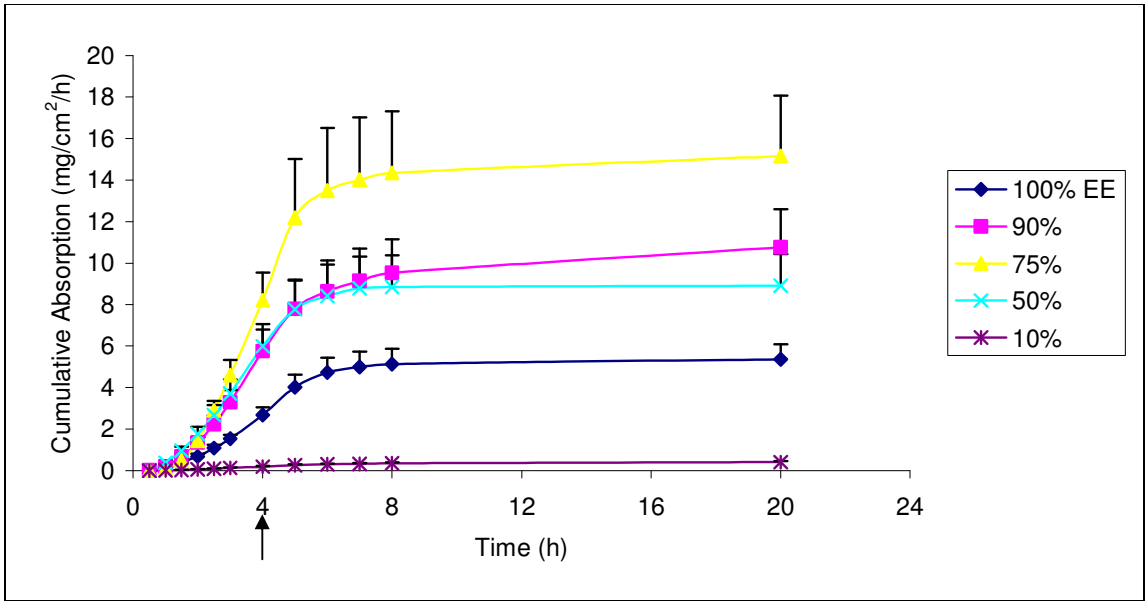
Table 6 Maximum penetration rates, apparent Kp and time to steady state of water from various mixtures through dermatomed human skin. Figures are mean ± SEM (n ≥ 5) *P< 0.05 when compared to water alone.

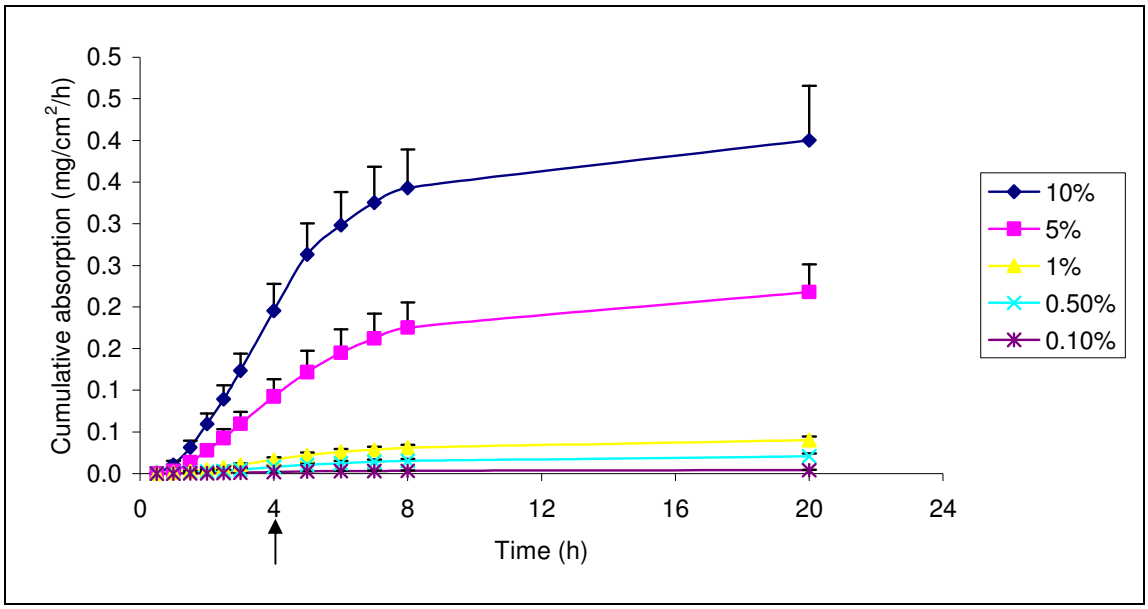
Concentration of water in test dose	Flux (mg/cm^2/h)	Kp (x10^{-3} cm/h)	Lag Time (h)
1000 mg/ml	4.72±0.52	4.71±0.52	0.84±0.04
500 mg/ml in butoxyethanol	5.17±0.70	10.34±1.40*	0.64±0.05
500 mg/ml in ethoxyethanol	5.28±0.40	10.56±0.80*	0.77±0.04
250 mg/ml in 1:1 butoxyethanol:ethoxyethanol	1.94±0.42	7.75±1.67	0.71±0.09


Cumulative Absorption (mg/cm2)
Time (h)
900ug/ml
810ug/ml
675ug/ml
450ug/ml
90ug/ml
45ug/ml
9ug/ml
4.5ug/ml
0.9ug/ml


Cumulative Absorption (mg/cm2)
Time (h)
900ug/ml
810ug/ml
450ug/ml


Percentage of Applied Dose
120
100
80
60
40
20
0
900 mg/ml
810 mg/ml
675 mg/ml
450 mg/ml
93 mg/ml
46.5 mg/ml
9.3 mg/ml
4.65 mg/ml
0.93 mg/ml
Receptor Fluid
Dose remaining on skin
Skin
Carbon Filters
Total


Cumulative Absorption (mg/cm2/h)
20
18
16
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
Time (h)
100% EE
90%
75%
50%
10%


Cumulative absorption (mg/cm2/h)
0.5
0.5
0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
0
4
8
12
16
20
24
Time (h)
10%
5%
1%
0.50%
0.10%

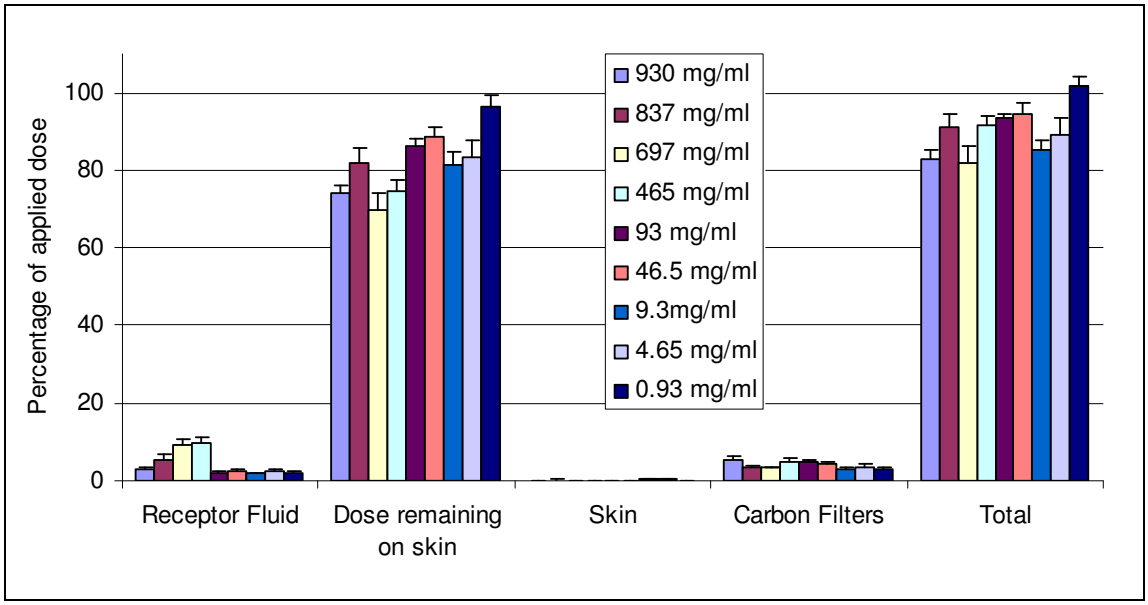

Percentage of applied dose
100
80
60
40
20
0
930 mg/ml
837 mg/ml
697 mg/ml
465 mg/ml
93 mg/ml
46.5 mg/ml
9.3mg/ml
4.65 mg/ml
0.93 mg/ml
Receptor Fluid
Dose remaining on skin
Skin
Carbon Filters
Total

Figure legends

Fig.1 Percutaneous absorption of butoxyethanol at various doses through dermatomed human skin in flow through diffusion cells. ↟ = dose removed. Results are means ± SEM ($n \geq 5$)

Fig 2 Percutaneous absorption of butoxyethanol at various doses through dermatomed rat skin in flow through diffusion cells. Results are means ± SEM ($n = 5$)

Fig 3 Balance of radioactivity for percutaneous penetration of butoxyethanol through dermatomed human skin. Results are means ± SEM ($n \geq 5$)

Fig 4 Percutaneous absorption of ethoxyethanol at various doses through dermatomed human skin in flow through diffusion cells. ↟ = dose removed. Results are means ± SEM ($n \geq 7$)

Fig 5 Percutaneous absorption of ethoxyethanol at various doses through dermatomed human skin in flow through diffusion cells. ↟ = dose removed. Results are means ± SEM ($n \geq 6$)

Fig 6 Balance of radioactivity for percutaneous penetration of ethoxyethanol through dermatomed human skin. Results are means ± SEM ($n \geq 6$)