Vehicle Effects on *in Vitro* Percutaneous Absorption Through Rat and Human Skin

Jennifer Hilton,[1] Bruce H. Woollen,[1] Robert C. Scott,[1] Timothy R. Auton,[1,2] Kathryn L. Trebilcock,[1] and Martin F. Wilks[1]

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We studied the effects of three vehicles (propylene glycol, octanol and ethyl decanoate) with differing polarity on the *in vitro* percutaneous absorption of three chemicals (fluazifop-butyl, dimethyl phthalate and fomesafen sodium salt) with a range of physico-chemical properties. Absorption rate measurements were made from high vehicle volume (200μl/cm^2) and low vehicle volume (<10μl/cm^2) applications. For the lipophilic fluazifop-butyl absorption rate was highest from the more polar vehicle propylene glycol, but this effect was only significant under high-volume conditions. There was a variable vehicle effect on absorption of the intermediate chemical dimethyl phthalate. The largest vehicle effect was seen for the more hydrophilic fomesafen sodium salt where absorption was fastest from the least polar vehicle ethyl decanoate. These results support the hypothesis that the absorption process can in part be predicted from a knowledge of solute solubility. Vehicle effects were greater from high volume applications than from those more comparable to occupational exposure conditions.

KEY WORDS: *in Vitro;* percutaneous absorption; vehicles; human; rat; skin.

INTRODUCTION

Many factors may influence the extent of percutaneous absorption of a chemical. One such factor is the vehicle in which it is applied to the skin (1–6). The rate of absorption of a chemical across the skin is believed to be dependent on the concentration in the vehicle (more correctly the chemical potential in the vehicle). Partitioning of the chemical between the vehicle and the stratum corneum (the outermost layer of the skin) results in a concentration gradient developing across the skin, which is influenced by chemical-vehicle-skin interactions (4). The primary interactions relevant to the penetrant and the vehicle are governed by the solubility of the penetrant and its diffusive mobility within the vehicle. Different vehicles may influence the absorption rate of a chemical by influencing the extent of partition into the stratum corneum, which is a consequence of the solubility of the penetrant in the vehicle (5). With a knowledge of the solubility of a penetrant in a vehicle, therefore, prediction and manipulation of absorption might be possible (6).

In the present study, *in vitro* percutaneous absorption of three model compounds was measured from a high volume vehicle application (200μl cm^{-2}) and from low (≤10μl cm^{-2}) volume applications. The high volume vehicle applications were used as these have been used commonly in many reported *in vitro* studies, and the low volume applications to more realistically mimic potential occupational exposure and predict *in vivo* absorption. Three model compounds were selected to provide a range of physico-chemical properties. It was anticipated that this would result in different solubilities of the chemicals in the vehicles (5). The most lipophilic compound was fluazifop-butyl (octanol:water partition coefficient Log P, 4.5). Dimethyl phthalate was chosen for its intermediate polarity (octanol:water Log P, 1.3). The most hydrophilic compound was fomesafen sodium salt (octanol:water Log P, −1.3).

In many dermal absorption studies a volatile vehicle such as acetone has been used to deliver the chemical to the skin (7). This approach, we believe, gives useful but limited information because the vehicle influences primarily the early phase of the absorption process, before it evaporates. Our study investigated the influence of (relatively) non-volatile vehicles on the absorption of the 3 test chemicals. The vehicles (propylene glycol, octanol and ethyl decanoate) were chosen to have a range of polarities (dielectric constants: 32, 10.3 and 2.9 respectively), similar volatility (to standardise the residence time on the skin surface) and low potential to damage skin (determined in our Laboratory and from literature references; data not included). The choice of vehicles was further restricted to those with low systemic toxicity as the vehicles were to be used in complementary human and animal *in vivo* studies.

The data presented describe the *in vitro* absorption of the three model compounds from each of the three vehicles. Absorption was measured through both human and rat skin in order to study any species differences in vehicle effects on the absorption process. This was considered important as the development of topical preparations and toxicological assessments often use animal models and the data are then extrapolated to man.

MATERIALS AND METHODS

Chemicals

Fluazifop-butyl (purity 97.4%) and [^{14}C] fluazifop-butyl (purity >96%, specific activity 1.931 GBq mmol^{-1}) were supplied by ICI Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK. Fomesafen sodium salt (purity 98.3%) and [^{14}C] fomesafen sodium salt (purity >95%, specific activity 1.854 GBq mmol^{-1}) were synthesized from the acid form of the compounds supplied by ICI Agrochemicals, Jealott's Hill Research Station. Dimethyl phthalate (purity >99%) was supplied by Aldrich Chemical Company, Dorset, UK. [^{14}C] dimethyl phthalate (purity >99%, specific activity 0.647 GBq mmol^{-1}) was synthesized from phthalic acid-carbonyl-C14, supplied by ICI Cambridge Research Biochemicals, Billingham, UK.

Test vehicles, propylene glycol (purity 99%), 1-octanol (purity 99.5%) and ethyl decanoate (purity >99%) were supplied by Aldrich Chemical Company, Dorset, UK.

[1] Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England.

[2] Address for correspondence: Dr T. R. Auton, Zeneca Central Toxicology Laboratory, Alderley park, Macclesfield, Cheshire SK10 4TJ, United Kingdom.

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Solubility Determination

The solubility of each compound in each vehicle was determined. Excess solute was added to the vehicle and mixed for 3 days at 25°C. After centrifugation the concentration of solute in the supernatant was determined by HPLC for fluazifop-butyl and fomesafen sodium salt and by GLC for dimethyl phthalate.

Epidermal Membrane Preparation

Alderley Park rats (strain Alpk:APfSD) were supplied by the Barriered Animal Breeding Unit, Alderley Park, Cheshire, UK and used in all studies involving fluazifop-butyl and fomesafen sodium salt. Sprague-Dawley rats (strain CrL:CD(SD)BR) were supplied by Charles River, UK, Ltd, Margate, Kent, UK and used in all studies involving dimethyl phthalate. Intact epidermal membranes from 29 day old rats were prepared by soaking whole skin in 1.5M sodium bromide for 18–20 hours, after which epidermal membranes could be peeled from the dermis (8).

Human epidermal membranes were prepared after immersion of human abdominal skin in water at 60°C for 45 seconds (8).

Following preparation all membranes were stored at 4°C for a maximum of 3 days.

Percutaneous Absorption Measurements

Prepared membranes were mounted on horizontal-membrane static glass diffusion cells (exposure area 2.54cm^2). The integrity of the membranes was assessed by measuring the permeability to tritiated water as described previously and any damaged membranes were rejected (8).

Dosing preparations (10mg ml^{-1}) of [^{14}C] labelled test material were prepared in each vehicle to allow application of between 4×10^6 and 1×10^8 dpm of radioactivity per cell to achieve detectable levels in the receptor fluids. The receptor chambers of the diffusion cells were filled with a known volume of 50% aqueous ethanol. This receptor fluid has previously been used in experiments and has been shown to give a good correlation between *in vivo* and *in vitro* absorption data for lipophilic penetrants (9). For all high volume experiments, 200μl cm^{-2} of dosing solution was applied to the surface of the epidermal membranes. Low volume applications of 5μl cm^{-2} to rat skin and 10μl cm^{-2} to human skin allowed complete coverage of the application area *in vitro*. Additional low volume applications to human skin of 1.25μl cm^{-2} were also assessed in order to mimic the actual application volume to be used in concurrent human volunteer studies. The absorption of the test chemicals from all three vehicles was assessed simultaneously to allow direct comparison of results. Between 5 and 9 diffusion cells (from 4–6 skin samples) were prepared for each of the vehicles tested. The cells were immersed in a water bath at 30 ± 1°C and the receptor solution stirred throughout the experiment. At intervals up to 54 hours after exposure 50μl samples were taken from the receptor fluid and the [^{14}C] radioactivity determined by scintillation counting after addition of 10ml of Optiphase scintillant (Fisons PLC, Loughborough, UK). The volume of the receptor fluid was maintained by addition of 50μl of fresh receptor fluid. The [^{14}C] content of the receptor fluid samples measured in dpm, was then converted to mg equivalents of test compound. From a graph of 'amount absorbed versus time', the steady-state rates of absorption were determined from the slopes of the curves.

Statistical Analysis

Statistical analysis was carried out using analysis of variance. Probabilities were calculated to determine when a significant effect of vehicle on maximum absorption rate occurred for a given chemical and experiment. A value of $p < 0.05$ was chosen as level of significance.

RESULTS

Solubility Determinations

The maximum solubility of each compound in each of the vehicles is given in Table I.

Measurement of Percutaneous Absorption Rates

The results show that for all compounds and at all application rates human skin is less permeable than rat skin. High volume applications of fluazifop-butyl to both human and rat skin demonstrated that absorption rates were slightly influenced by the vehicle used (Tables 2 and 3). The absorption rates decreased as vehicle polarity decreased. In both cases analysis of variance confirmed a small but significant difference. In the case of dimethyl phthalate, high volume applications to human skin gave faster absorption rates from the propylene glycol and ethyl decanoate vehicles than from octanol (Table 2). However high volume applications to rat skin showed a trend towards increased absorption rates as the polarity of the vehicle decreased (Table 3). The absorption rate of fomesafen sodium salt was greatly influenced by dosing vehicle. The rates increased markedly as the vehicle polarity decreased (Tables 2 and 3).

Low volume applications of fluazifop-butyl to human skin showed no significant vehicle effect on the rate of absorption of the compound (Table 2). Vehicle did not significantly affect the absorption of dimethyl phthalate at 1.25μl cm^{-2} and only a slight effect was seen at 10μl cm^{-2} (Table 2). The absorption of fomesafen sodium salt was clearly affected by the dosing vehicle (Table 2). A comparison between absorption rate from the lowest volume (1.25μl cm^{-2})

Table I. Maximum Solubility Measurements (mg/ml) of the Test Compound in the Vehicles

Compound	Vehicle		
	Propylene glycol	Octanol	Ethyl decanoate
Fluazifop-butyl	38	>600†	>600†
Dimethyl phthalate	541	>600†	>600†
Fomesafen sodium salt	631	12	10

Concentrations in mg/ml of compound in vehicle as determined by HPLC in the case of fluazifop-butyl and fomesafen sodium salt and by gas chromatography in the case of dimethyl phthalate.

† It was technically impractical to determine the solubility any more accurately than the value shown.

Table II. Maximum Absorption Rates ($\mu g \cdot cm^{-2} h^{-1}$) ± s.e.m. (n = 5–9) for Fluazifop-Butyl, Dimethyl Phthalate, and Fomesafen Sodium Salt through Human Epidermal Membranes

Application volume ($\mu l \cdot cm^{-2}$)	Application† vehicle	Penetrant					
		Fluazifop-butyl		Dimethyl phthalate		Fomesafen sodium	
200	PG	0.39 ± 0.07	ooee††	2.5 ± 0.06	o	0.021 ± 0.009	
	Oct	0.099 ± 0.02		1.4 ± 0.21		0.57 ± 0.22	
	ED	0.064 ± 0.007		2.5 ± 0.29	o	21 ± 4.0	ppoo
10	PG	0.17 ± 0.04		0.81 ± 0.09		0.008 ± 0.003	
		0.18 ± 0.04		0.98 ± 0.09	ee	0.22 ± 0.09	
		0.12 ± 0.03		0.65 ± 0.06		1.4 ± 0.12	ppoo
1.25	PG	0.045 ± 0.01		0.17 ± 0.02		0.001 ± 0.0004	
		0.019 ± 0.004		0.24 ± 0.03		0.004 ± 0.001	p
		0.02 ± 0.004		0.25 ± 0.02	p	0.013 ± 0.002	ppoo

† PG = Propylene Glycol, Oct = Octanol, ED = Ethyl Decanoate.
†† e, o, p, (ee, oo, pp) = Significantly greater at $p < 0.05$ ($p < 0.01$) than absorption from ethyl decanoate, octanol or propylene glycol vehicle, respectively.

application compared with the next higher volume (10μl cm^{-2}) shows that the vehicle effect seen for fomesafen was much more pronounced from the higher volume application. It should be noted that in the experiments with fomesafen at 1.25μl/cm^2 in human skin with propylene glycol significant absorption occurred in the period 0 to 4 hours. This high initial rate was not sustained in steady-state.

Low volume application to rat skin produced no significant vehicle effect on the absorption rate of fluazifop-butyl (Table 3). In common with the high volume application, the absorption rate of dimethyl phthalate increased as the vehicle polarity decreased after low volume applications to rat skin. The absorption of fomesafen sodium salt was again affected by the dosing vehicle used (Table 3).

DISCUSSION

Many of the published studies examining the influence of vehicles on absorption have been carried out *in vitro* under theoretically 'ideal' infinite volume application conditions (10–15). In most of these studies the solubility of the solute in the vehicle has been shown to affect absorption.

Fewer *in vitro* studies have been carried out under conditions which mimic *in vivo* exposure levels. In one example (15), the absorption of benzoic acid, caffeine and testosterone was measured through human skin from three vehicles. The derived permeability coefficients for each compound in each vehicle correlated with the percentage saturation, which reflects the solubility, demonstrating that the vehicle influenced the absorption process.

The results from our *in vitro* studies, particularly under high volume 'ideal' absorption conditions, support the finding from previous work that the percutaneous absorption of a chemical can be influenced by its concentration and the vehicle in which it contacts the skin. Previous studies have also shown that the effect of vehicles on the absorption process could in part be predicted from a knowledge of solute solubility (thermodynamic activity) in the vehicle (16,17). Theoretically, maximum absorption rates should occur from saturated solutions and below saturation the absorption rates should be proportional to the degree of saturation (18).

With fluazifop-butyl (our most lipophilic test compound) the high volume applications resulted in the highest rate of absorption from the propylene glycol vehicle. The com-

Table III. Maximum Absorption Rates ($\mu g \cdot cm^{-2} h^{-1}$) ± s.e.m. (n = 5–9) for Fluazifop-Butyl, Dimethyl Phthalate, and Fomesafen Sodium Salt through Rat Epidermal Membranes

Application volume ($\mu l \cdot cm^{-2}$)	Application† vehicle	Penetrant					
		Fluazifop-butyl		Dimethyl phthalate		Fomesafen sodium	
200	PG	5.2 ± 0.86	ee††	4.0 ± 0.41		0.084 ± 0.02	
	Oct	3.5 ± 0.46	ee	31 ± 0.79	pp	27 ± 1.4	pp
	ED	0.85 ± 0.045		51 ± 2.3	ppoo	560 ± 44	ppoo
10	PG	2.0 ± 0.26		Not determined		Not determined	
	Oct	2.0 ± 0.28		Not determined		Not determined	
	ED	2.0 ± 0.33		Not determined		Not determined	
5	PG	1.0 ± 0.18		5.9 ± 0.84		0.074 ± 0.02	
	Oct	1.0 ± 0.11		7.7 ± 0.23	p	1.9 ± 0.20	pp
	ED	0.72 ± 0.25		13 ± 0.84	ppoo	4.0 ± 0.44	ppoo

† PG = Propylene Glycol, Oct = Octanol, ED = Ethyl Decanoate.
†† e, o, p, (ee, oo, pp) = Significantly greater at $p < 0.05$ ($p < 0.01$) than absorption from ethyl decanoate, octanol or propylene glycol vehicle, respectively.

pound is at a higher degree of saturation (as it has lower solubility) in propylene glycol than in the other vehicles; this could account for the finding. However, the absorption rates measured were not directly proportional to the degree of saturation. If this were the case, the absorption rate from the propylene glycol would be predicted to be at least 15 fold faster than from the other two vehicles, as judged from the solubility measurements. In reality a smaller vehicle effect was measured which varied with application volume and skin type. From low application volumes the absorption rate of the fluazifop-butyl was not significantly influenced by the vehicle. This fact demonstrates that, under exposure conditions which are closer to those experienced occupationally, absorption of this lipophilic test compound is unlikely to be greatly affected by the delivery vehicle.

The absorption rate of dimethyl phthalate, which has intermediate solubility properties, through human skin does not appear to be influenced by the vehicle. This is in agreement with the prediction made from the solubility measurements (see Table I). There is a significant vehicle effect through rat skin where the absorption rates are faster, but this cannot be explained by the small differences in solubility of this compound in the three vehicles. The influence of solubility would be expected to favour slightly faster absorption of the compound from the more polar vehicle propylene glycol in which it is less soluble. This is, however, difficult to quantify since the solubility measurements of dimethyl phthalate in the other two vehicles do not permit an exact assessment of the true differences. In practice, the absorption rate through rat skin was faster from the ethyl decanoate vehicle. The magnitude of difference between absorption from the three vehicles was less following low-volume application than for high-volume application.

The largest vehicle effect was demonstrated with fomesafen sodium salt, the most hydrophilic compound. The absorption rate was fastest from the non-polar vehicle ethyl decanoate and decreased progressively in the octanol then propylene glycol vehicles. The differences in absorption rates from the vehicles were more pronounced for high volume applications than for low volume applications across both rat and human skin. These findings can also be explained in terms of solubility of the compound. The lower the solubility in the vehicle the greater the potential for the compound to partition out of the vehicle and into the skin, leading to the establishment of a high concentration in the outer stratum corneum and thus a marked concentration gradient which favours faster absorption of the compound.

It is interesting to speculate on the general implications of our findings for other chemicals for both pharmaceutical and toxicological scenarios. Two hypotheses emerge from these studies. First the data suggest that vehicle effects may be more pronounced for more water soluble penetrants. Second chemicals with intermediate log P values appear to be more favourably absorbed regardless of the vehicle. This seems to be particularly true for applications which are closer to occupational exposure conditions, as shown in Table 2, where the compound with the intermediate log P had by far the highest absorption rate. Whether these considerations are applicable to other chemicals and vehicles can only be shown by further experiments.

In summary, the theoretical predictions of vehicle influence on the rate of absorption based on the solubility of the compounds in the vehicles are essentially supported by the experimental data generated under high volume application conditions. From low volume applications, which are closer to occupational and pharmacological exposure conditions, the absorption of the compounds is influenced to a lesser extent by the choice of delivery vehicle.

The influence of application volume and vehicle on skin penetration are considered in more detail in a companion paper (19) which describes the derivation and validation of a mathematical model relating penetration rate to the applied volume and concentration. Vehicle effects in the model are explained in terms of partition coefficients of the penetrants in the different vehicles.

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