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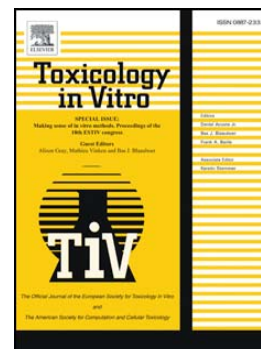
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**Further development of an *in vitro* model for studying the penetration of chemicals
through compromised skin**

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Abbreviations: ER, Electrical Resistance; LSC, Liquid Scintillation Counting; SCCS, Scientific Committee on Consumer Safety; EFSA, European Food Safety Authority. OECD, Organisation for Economic Co-operation and Development.

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Abstract – A new *in vitro* model based on the electrical resistance properties of the skin barrier has been established in this laboratory. The model utilises a tape stripping procedure in dermatomed pig skin that removes a specific proportion of the *stratum corneum*, mimicking impaired barrier function observed in humans with damaged skin. The skin penetration and distribution of chemicals with differing physicochemical properties, namely; Benzoic Acid, 3-Aminophenol, Caffeine and Sucrose has been assessed in this model. Although, skin penetration over 24 h differed for each chemical, compromising the skin did not alter the shape of the time course profile, although absorption into receptor fluid was higher for each chemical. Systemic exposure (receptor fluid, epidermis and dermis), was marginally higher in compromised skin following exposure to the fast penetrant, Benzoic acid, and the slow penetrant Sucrose. The systemically available dose of 3-Aminophenol increased to a greater extent and the absorption of Caffeine was more than double in compromised skin, suggesting that Molecular Weight and Log P_{ow} , are not the only determinants for assessing systemic exposure under these conditions. Although further investigations are required, this *in vitro* model may be useful for prediction of dermal route exposure under conditions where skin barrier is impaired.

Keywords: Dermatomed pig skin; Electrical Resistance (ER); Barrier function; Compromised skin, Tape stripping; *In vitro* percutaneous absorption

1. INTRODUCTION

The assessment of the dermal absorption potential of ingredients in consumer, personal care, pharmaceutical and industrial chemical products that come into contact with the skin is a key part of human risk assessment. This ensures that under normal or expected conditions, that there is a sufficient margin of safety for manufacturers, handlers and end users of products containing a particular chemical. Dermal absorption is now almost exclusively determined using *in vitro* techniques and regulatory studies used for human risk assessment follow the *in vitro* OECD 428 test guideline using human or animal skin that has normal intact barrier properties. This approach is used extensively by the personal care/cosmetic, biocide and agrochemical industries, together with specific guidance for the type of product used, in order to predict human exposure *via* the dermal route (EFSA, 2012; SCCS, 2010). These *in vitro* protocols form part of the registration process that ensure the safety of new and existing products that come into contact with the skin either intentionally or during their occupational use.

The OECD 428 test guideline has been used now for more than a decade as a stand-alone *in vitro* model for dermal absorption assessment. Current regulations for consumer products and chemicals use industry-specific protocols for dermal penetration. Each of these guidance documents assume that the product will only come in contact with healthy, intact human skin (EFSA, 2012; SCCS, 2010). However, there are product applications and consumer needs that involve potentially compromised skin and also industrial products may be used by individuals with compromised skin. This research is aimed to bridge that gap by generating quantitative evidence of the extent of the difference between normal and compromised skin. There are a number of previously published studies in this area that have used models of mechanically or chemically-damaged skin to determine quantitative changes in dermal exposure under such conditions (Dey, et al., 2014; Kezic

and Nielson, 2009;). Although the damage to the skin barrier is quite different in conditions such as psoriasis or eczema compared with topical damage to the stratum corneum, similar *in vitro* approaches to that described in this investigation have also been utilised for chemicals used to treat skin diseases (Chiang et al., 2011; Goon et al., 2004; Jakasa et al., 2006). However, these previous investigations were designed for specific scenarios and for specific chemical classes. Furthermore, previous investigations using compromised skin have focused on the absorption of chemicals into the receptor fluid only, without considering the other regions of the skin beyond the *stratum corneum* that contribute to the “systemically available” dose in regulatory studies. Therefore, in this investigation we have also included the mass balance distribution of various reference chemicals in both normal and compromised skin, bringing it in line with modern day guidance used to estimate dermal absorption.

A previous investigation in our laboratory published in this journal evaluated the potential of a new *in vitro* compromised skin model using a tape stripping technique in pig skin (Davies et al., 2015). The objective of this previous research was to provide an *in vitro* experimental approach that could be used to investigate the dermal absorption of consumer products when the skin barrier was impaired (Davies et al., 2015), but distinct from fully abraded skin for which there is no *stratum corneum* barrier. Assessment of dermal absorption through an impaired skin barrier is particularly important for certain products such as chaffed, cracked skin protectants, sunscreens and diaper rash creams, since they are often applied to the skin after the *stratum corneum* has already been damaged either by UV exposure or irritated by biological fluids in diaper dermatitis (Stamatas et al., 2011). There is little knowledge as to the impact of such damage on dermal uptake for such products. The same could be argued for industrial chemicals and pesticides where they may be handled or sprayed by workers with damaged skin or by

individuals with skin conditions such as dermatitis, eczema or psoriasis. We therefore set out to further develop a practical *in vitro* model of skin damage by tape stripping that was published recently in this journal (Davies et al., 2015). In the previous investigation we had determined that a tape stripping regime that involved 10 sequential strips changed the barrier properties of the skin to a level that was equivalent to skin barrier damage in man, as assessed by the non-invasive integrity assessment, transepidermal water loss.

In this follow up investigation we have evaluated how robust our model is and how useful it could be in a risk assessment context for the safety of chemicals that are used on damaged skin either intentionally or accidentally. The *in vitro* skin model has been evaluated using a number of reference chemicals that represented a range of molecular weights and polarities. The chemicals selected for this exercise were Benzoic Acid, 3-Aminophenol, Caffeine and Sucrose. We have compared the dermal absorption properties of several of these reference chemicals in human skin in previous studies and across different laboratories (Heylings et al., 2007). The purpose of this study was, therefore, to investigate the skin penetration and skin distribution of these chemicals in normal and compromised pig skin, based on the guidance for assessing systemic exposure *via* the dermal route, as used by the Scientific Committee on Consumer Safety for cosmetic ingredients (SCCS, 2010) and European Food Safety Authority for pesticide-containing products (EFSA, 2012). Both these industry groups base their guidance on the principles described in OECD test guideline 428 for *in vitro* dermal absorption (OECD, 2004a).

2. METHODS

2.1 Preparation of dermatomed skin membranes

Pig skin preparations used for this investigation were from animals of the British White strain of pig (aged 6-8 weeks) that had been bred for food and were sourced from a local abattoir. Pig skin is a predictive model for human skin penetration since it has very similar morphology and permeability properties to human skin (Dick and Scott, 1992) and it is permitted in regulatory studies for the dermal penetration of cosmetic ingredients (SCCS, 2010). Skin membranes (approximately 6 cm diameter) were cut at a thickness of 200-400 μm using an electric dermatome. Each skin membrane was given a unique identifying number and stored frozen, at -20°C , on aluminium foil, until required for use.

2.2 In vitro static diffusion cell equipment and measurement of skin integrity

Details of the diffusion cell assembly used in these investigations are described in the OECD Guideline 428 (OECD, 2004a) and Guidance Document No. 28 (OECD, 2004b). Discs of dermatomed pig skin approximately 3.3 cm diameter were mounted dermal side down in Franz-type glass static diffusion cells (Dugard et al., 1984; Franz, 1975; Scott and Clowes, 1992). Each cell had an exposed area of skin of 2.54 cm^2 . The receptor chambers were filled with a recorded volume of physiological saline and placed on a magnetic stirrer plate in a water bath maintained at a skin temperature of $32 \pm 1^{\circ}\text{C}$. Prior to use, the skin integrity of each skin membrane was assessed using Electrical Resistance (ER) and the cut-off criteria established previously in our laboratory (Davies et al., 2004; Heylings et al., 2001). Resistance across each membrane was measured using a PRISM Electronics AIM6401 LCR data bridge and was expressed as $\text{k}\Omega/\text{cell area}$. This method measures the resistance or impedance of skin samples in diffusion chambers and has been shown by several laboratories to be representative of skin barrier function (Davies et al., 2004; Lawrence et al., 1997; White et al., 2011). The chambers were allowed to equilibrate in a water bath at 32°C for approximately 30 minutes after-which electrodes

were inserted into saline in the receptor chamber side arm and into the saline in the donor chamber. Once stabilised, the resistance value was recorded. Any skin membranes that had an ER value below 3 k Ω , a cut off value described previously for pig skin (Davies et al., 2004), were not used in these investigations. The remaining membranes, from a maximum of 5 animals, were split randomly into two groups of 12 cells per reference chemical. To ensure that anomalies were not introduced into these investigations, skin membranes from the same 5 animals were used throughout. These intact skin membranes were randomly distributed between two groups of diffusion cells. One group represented 'normal' skin and the other group represented 'compromised' skin, following the 10 x tape stripping routine described previously (Davies et al., 2015). The tape stripping method followed the standard approach described in test guideline OECD 428 (Trebilcock et al., 1994), using 22 mm diameter CuDerm D-Squame stripping discs (CuDerm Corporation, Dallas, USA) which were applied to the dry skin surface at a constant pressure of 225 g/cm² for 5 seconds, using a purpose-built applicator.

2.3 Application of the reference chemicals

The reference chemicals used were Benzoic acid, 3-Aminophenol, Caffeine and Sucrose (Table 1). After completion of the skin stripping procedure, the receptor chambers of the cells ('normal' and stripped) were filled with a recorded volume of physiological saline receptor fluid. For each application, a mixture of unlabelled and [¹⁴C]-Benzoic acid, [¹⁴C]-3-Aminophenol, [¹⁴C]-Caffeine and [¹⁴C]-Sucrose were each separately formulated in basic emulsion bases and water to achieve a concentration of 10 mg/mL of each compound with a radioactivity content of approximately 1.5 MBq/mL. Finite applications (10 μ L/cm²) were applied to the surface of the skin membranes at a dose of 100 μ g/cm²

and left on the surface of the normal and compromised skin membranes for 24 h. The diffusion cells were placed semi-immersed in a water bath maintained at $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Samples (0.5 mL) of the receptor fluid were taken manually immediately before dosing and then at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h after application, using an auto-sampler. The radioactivity content of the receptor fluid samples was determined by LSC analysis following the addition of scintillation fluid. The volume of fluid in the receptor chamber was maintained by the replacement of a volume of receptor fluid, equal to the sample volume immediately after each sample was taken. After 24 h, the donor chamber was carefully detached from the cell assembly after which a mild skin washing procedure was performed which involved the swabbing of the skin surface firstly with sponges pre-wetted with a dilute soap solution and then sponges pre-wetted with water. The flange skin (the unexposed/undosed skin which had been held between the donor and receptor chamber) was cut away and the epidermis was separated from the dermis by heat separation. The skin tissues were solubilised with tissue digestant prior to analysis by LSC.

2.4 *Calculations and statistical analyses*

The time-course absorption into the receptor fluid was calculated by dividing the amount of test penetrant at each time point by the area of exposed dermatomed skin (2.54 cm^2) and plotting the results as amount of penetrant absorbed ($\mu\text{g}/\text{cm}^2$) versus time (h). The slope of the absorption profile between given time points provided the average rate of absorption of the penetrant per cm^2 of the skin ($\mu\text{g}/\text{cm}^2/\text{h}$) during that period. The proportion ($\mu\text{g}/\text{cm}^2$) of test penetrant that was recovered from the individual compartments of the system (donor chamber, skin wash, flange, epidermis, dermis and receptor fluid) was expressed as a percentage of the total amount applied to the surface of the skin

membrane in order to establish the mass balance distribution of the chemical in all the experimental compartments. The normal and compromised skin values were expressed as mean \pm SEM for each group. A comparison was made between unstripped 'normal' skin and 'compromised' skin using Student's *t*-test for unpaired variates.

3. RESULTS

3.1 Skin Penetration of Benzoic acid, 3-Aminophenol, Caffeine and Sucrose

Benzoic acid gave the highest actual values for skin penetration into the receptor fluid over 24 h in both normal and compromised skin with Sucrose giving the lowest values for the reference chemicals (Figure 1 and Table 2). The shapes of the time course profiles for each compound were quite similar between normal skin and compromised skin (Figure 2). However, the skin penetration of each compound into the receptor fluid was higher in compromised skin in each case. This trend was reflected across the four chemicals when the absorption through the skin was expressed as either % of the dose penetrated or as an average rate of absorption or flux (Table 2). Interestingly, although the skin barrier integrity had been compromised to an extent that more test chemical penetrated through the skin membrane, the "damaged" membranes still retained significant skin barrier capabilities, restricting the access of the penetrant to the receptor fluid beneath the skin. This was in line with previous results of Davies et al (2015) in which this tape stripping regime was developed to model barrier function of common skin conditions.

With regard to the magnitude of the change in skin penetration, based on the total proportion of the dose reaching the receptor fluid at 24 h, Benzoic acid, 3-Aminophenol, Caffeine and Sucrose gave about a 1.5-2.5 fold increase through compromised skin, when compared to their normal skin control group. This was statistically significant for Benzoic

acid ($p < 0.0001$), 3-Aminophenol ($p < 0.01$) and Caffeine ($p < 0.01$). Although a 1.5 fold increase absorption of Sucrose was noted through compromised skin, this was not statistically significant ($p > 0.05$).

The relative absorption rates of the compounds demonstrated the same association between normal and compromised skin. Benzoic acid increased from $1.42 \mu\text{g}/\text{cm}^2/\text{h}$ in normal skin to $1.99 \mu\text{g}/\text{cm}^2/\text{h}$ in compromised skin. The average skin flux for 3-Aminophenol increased from $0.67 \mu\text{g}/\text{cm}^2/\text{h}$ in normal skin to $1.05 \mu\text{g}/\text{cm}^2/\text{h}$ in compromised skin. For Caffeine, the average absorption rate increased from $0.21 \mu\text{g}/\text{cm}^2/\text{h}$ in normal skin to $0.51 \mu\text{g}/\text{cm}^2/\text{h}$ in compromised skin. The absorption rate of Sucrose was similar between the two skin models with average absorption rates between 0-24 h being $0.014 \mu\text{g}/\text{cm}^2/\text{h}$ for the normal skin and marginally higher at $0.021 \mu\text{g}/\text{cm}^2/\text{h}$ through compromised skin.

3.2 Skin Distribution of Benzoic acid, 3-Aminophenol, Caffeine and Sucrose

The OECD test guideline 428 for the assessment of dermal absorption *in vitro* also requires that dermal absorption to be qualified by the inclusion of the mass balance distribution of the compound of interest (OECD, 2004). Each of the regulatory authorities such as SCCS and EFSA in Europe specify which compartments of the mass balance are regarded as “dermally absorbed” or “potentially absorbed” for the test chemical being investigated in their risk assessment processes (EFSA, 2012; SCCS, 2010). An important consideration in this investigation was to examine where the applied dose of the various reference compounds were located at the 24 h time point following application to both normal skin and compromised skin.

The total mass balance recoveries were very good with all the reference compounds giving values close to 100% of the dose applied being recovered in the

various compartments. This ranged from 97.8% for Benzoic acid to 104% for Caffeine in normal skin and 102% for Benzoic acid to 107% for Caffeine in compromised skin. The full mass balance skin distribution of each of the compounds in the two skin types is shown in Figure 3 and Tables 3 and 4.

It was noted that a considerable proportion (34%) of the applied Benzoic acid was lost from the skin surface, due to its low molecular weight, into the head space above the skin membrane which was maintained at $32 \pm 1^\circ\text{C}$ over 24 h. An almost identical amount of Benzoic acid was lost to the atmosphere from compromised skin. Unsurprisingly, since normal skin has an intact *stratum corneum*, more of the Benzoic acid dose was available for removal by washing the skin surface (20%) of normal skin at 24 h compared with 10% of the applied dose in compromised skin. Similar and lower proportions of the Benzoic acid dose were found in the remaining epidermis and dermis skin in both skin types, but significantly more of this compound actually reached the receptor fluid (51% versus 35%) in compromised skin.

3-Aminophenol gave an interesting distribution profile in the skin in the mass balance experiments. Despite much of the *stratum corneum* having been removed in the compromised skin group, about the same proportion of the dose (73-75%) could still be removed by surface skin washing at 24 h. Similar amounts of 3-Aminophenol were found in the epidermis and dermis in the two skin groups. However, the reduced barrier properties of compromised skin allowed a significantly higher proportion of the dose (25%) to reach the receptor fluid at 24 h compared with 16% in normal skin. The lower molecular weight and more polar 3-Aminophenol (LogP_{ow} 0.18) gave similar receptor fluid profiles in normal and compromised skin with a short lag phase of around an hour after which absorption steadily increased.

The more hydrophilic Caffeine penetrated the skin very slowly and, after a small lag phase of about half an hour, there was a gradual increase in absorption into the receptor phase through both normal and compromised membranes. The vast majority (over 91%) of the dose applied was removed from the surface of both normal and compromised skin at 24 h. Similar low amounts of the dose were found in the epidermis and dermis in the two groups. The only discernible difference in mass balance distribution with Caffeine occurred with the receptor dose which was higher in compromised skin at 13% compared with only 5% in normal skin.

Sucrose has physicochemical properties that make it a poor skin penetrant, being quite hydrophilic and having a relatively high molecular mass. Overall, the perturbation in the skin barrier by tape stripping had virtually no effect on the skin penetration or distribution of topically applied Sucrose. Indeed, practically all of the applied Sucrose was still present on the skin surface after 24 h with 99.8% of the applied dose being removed by mild skin washing. This left only trace amounts of Sucrose in the epidermis and dermis (less than 1% of the applied dose). Although very little Sucrose penetrated the skin, the compromised skin group still showed slightly increased absorption at 24 hours (0.6% of the applied dose) compared with 'normal' skin (0.4%).

4. DISCUSSION

This investigation is a follow up article to a previous recent publication in this journal where we developed a standardised *in vitro* model to study percutaneous absorption through compromised skin (Davies et al., 2015). We had specifically developed this *in vitro* model since much less is known about the ability of chemicals to permeate through the skin when the *stratum corneum* barrier is physically damaged but not completely absent. Very little is known about the skin distribution of chemicals under such

circumstances. Since the localisation of chemicals within the skin layers is an important area of human risk assessment we have refined our *in vitro* model to include a mass balance distribution of various reference chemicals in compromised skin. This is an important part of human risk assessment since consumer and personal care products may be used by individuals who have a compromised skin barrier (Goon et al., 2004) or in situations such as diaper dermatitis, where infants may have irritated skin (Stamatas et al., 2011). Therefore, it is useful to assess the impact this has for different chemicals and how it may change the assessment of the bioavailable fraction of the dose applied. Furthermore, topical exposure to other industrial chemicals such as pesticides during their intended use may also involve contact with damaged skin and an understanding and characterisation of dermal absorption and dermal distribution under such conditions is an important consideration from a safety perspective. Our tape stripped dermatomed pig skin model has been shown to mimic the conditions of skin damage observed in the clinic, as assessed by trans-epithelial water loss (Davies et al., 2015), a non-invasive barrier function measure that can be used to assess barrier function in man (Imhof et al., 2009; Kim et al., 2006). One distinct benefit of our proposed model incorporating compromised skin is that it does not involve any animal testing, unlike previous models in this area where chemicals have been applied to skin membrane of living animals where the tissue has been mechanically abraded.

The *in vitro* approach as described in test guideline OECD 428 has been used successfully for many years to predict dermal absorption of chemicals in man. We have explored whether this *in vitro* protocol can be adapted to mimic the circumstances encountered every day such as the application of consumer products to damaged or irritated skin. One example of this is the use of sunscreen products, many of which contain UV absorbers such as zinc oxide and titanium dioxide. Although it should be

recognised that some of these products may be presented to the skin as nano materials rather than organic molecules dispersed in a formulation (SCCS, 2012). Sunscreen products are often used on sunburnt skin which, of course, is likely to have reduced barrier properties, yet little is known about how the systemic availability of these chemicals may be altered under these compromised skin conditions.

The present study has further explored the *in vitro* compromised skin model including an assessment of mass balance distribution, using a range of chemicals with different molecular weights and different octanol:water partition coefficients (LogP_{ow}). Topical application of formulations containing Benzoic acid, 3-Aminophenol, Caffeine and Sucrose to normal undamaged skin, and to skin compromised by tape stripping, showed that the physicochemical properties that influence dermal absorption in normal skin do, to a large extent, still hold for compromised skin in our model. The physicochemical properties of molecular size and polarity and their ability to predict the rate of dermal absorption generally prevail. For example, Benzoic acid with its relatively low molecular weight and LogP_{ow} of 1.87 are physicochemical features consistent with good skin penetration. In contrast, the much higher molecular weight and water soluble nature of Sucrose gave consistently low skin penetration values in both skin types.

An interesting observation here was the reproducibility of the effects across all the test penetrants. We expected a much higher degree of variance in the compromised skin group. However, the robustness of the absorption and distribution data for each chemical in compromised skin was remarkably consistent across the twelve replicates in each group and for each chemical. This is probably due to a consistency in the tape stripping process itself leading to a uniform model of compromised skin, as we have shown previously in this laboratory (Davies et al., 2015).

The absorption of the chemicals through the skin into the receptor fluid, often presented as a skin flux or absorption rate are common parameters used in the dermal absorption field. However, the *in vitro* skin models that follow OECD test guideline 428 and are used in risk assessment to derive a predicted systemic dose of a chemical from its formulated product also now require the mass balance distribution of the applied finite (low volume) dose of the test chemical to be reported in order to validate the study. The fraction of the dose applied that has reached the bioavailable areas of the epidermis and dermis is added to the receptor dose to give the predicted amount of the test chemical under investigation that is “systemically available”. A key study performance criterion in OECD test guideline 428 for *in vitro* dermal absorption assessment (OECD, 2004a) is that the mass balance recovery should be 100 +/- 10% of the dose applied. Any missing dose unaccounted for in the mass balance is often presumed to be dermally absorbed due to the conservative nature of human risk assessment. In this investigation, the total recoveries were very good and this was achieved for all the chemicals tested in both normal and compromised skin. As shown in Figure 3, there was an inverse relationship between the amount washed off the skin at 24 h and the receptor fluid penetrated dose for 3-Aminophenol, Caffeine and Sucrose. However, for Benzoic acid, a significant proportion of the dose was lost by volatility and a high proportion of the applied dose penetrated both normal and compromised skin into the receptor fluid. Interestingly, from a systemic availability perspective, it was not the relatively fast skin penetrant Benzoic acid that showed the greatest increase in total systemic exposure, it was the physicochemical attributes of Caffeine that gave a 121% increase in predicted systemic availability in the compromised skin model compared with only a 35% increase with Benzoic acid. Although we have only studied a small number of chemicals under these controlled conditions, these investigations have alerted us to the fact that some compounds, with what may be

regarded as intermediate skin penetration characteristics, may actually have the physicochemical attributes that make them more prone to greater increases in dermal bioavailability in compromised skin. In contrast, chemicals such as 3-Aminophenol and Sucrose that may have previously been considered to be likely candidates for higher exposure under these damaged skin conditions by virtue of their physicochemical properties did not show a great deal of enhanced dermal absorption through compromised skin.

This investigation on dermal absorption through normal and compromised skin involved testing of the actual chemical substance formulated in a basic emulsion base and kept all the other conditions of application concentration, dose volume and radioactivity the same. However, it should be noted that the presence of other constituents in a finished product, particularly surfactants and emollients or other constituents present to aid solubility and dispersion, or even penetration into or through lipid type membranes, if it forms part of a personal care product, can also have an influence on the skin penetration and systemic exposure of the active ingredient. The objective of this investigation was to identify areas where a study in compromised skin may be a useful exercise to not only identify an unknown risk to human health, *via* increased dermal exposure, but also to preserve the approved use of a product where unnecessary restrictions may have been placed on it in a risk assessment, when it might be perfectly safe to use even on compromised skin.

More work is needed on a wider range of chemicals and conditions of study beyond this “proof of concept” investigation presented here, in order to provide further information on how the margin of safety should be considered alongside the intended use of specific consumer or personal care products. In the dermatological products area, this type of detailed skin distribution data for different chemicals that can be gained *in vitro* using

normal and compromised skin could be useful to optimise the beneficial actions of new topical formulations. However, we believe that the most useful application of this compromised skin model is in the risk assessment area for industrial chemicals, where major and arbitrary adjustments to the margin of safety are often made on the basis that the chemical may come in contact with human skin with low barrier properties.

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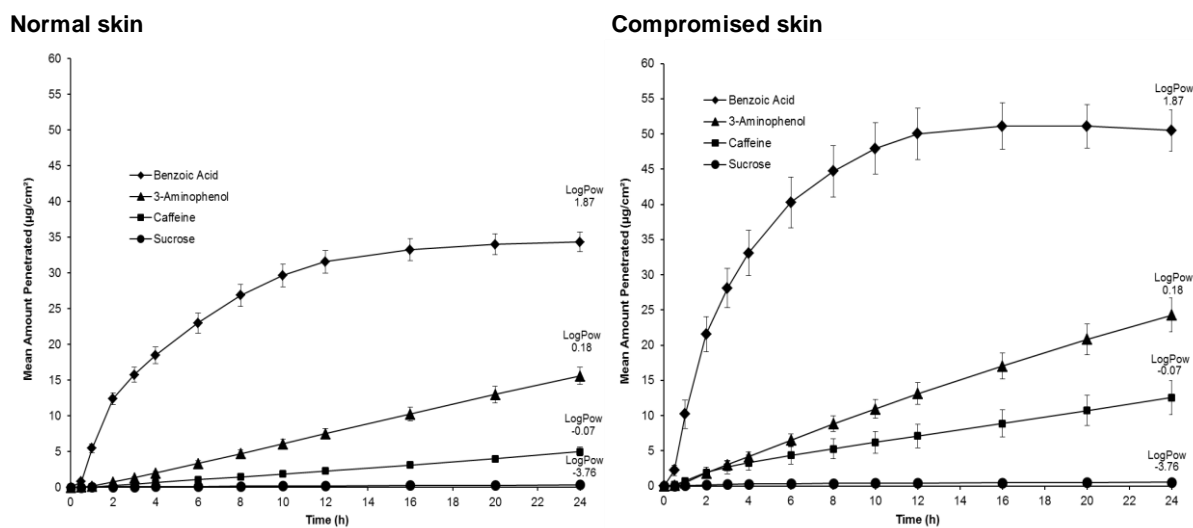
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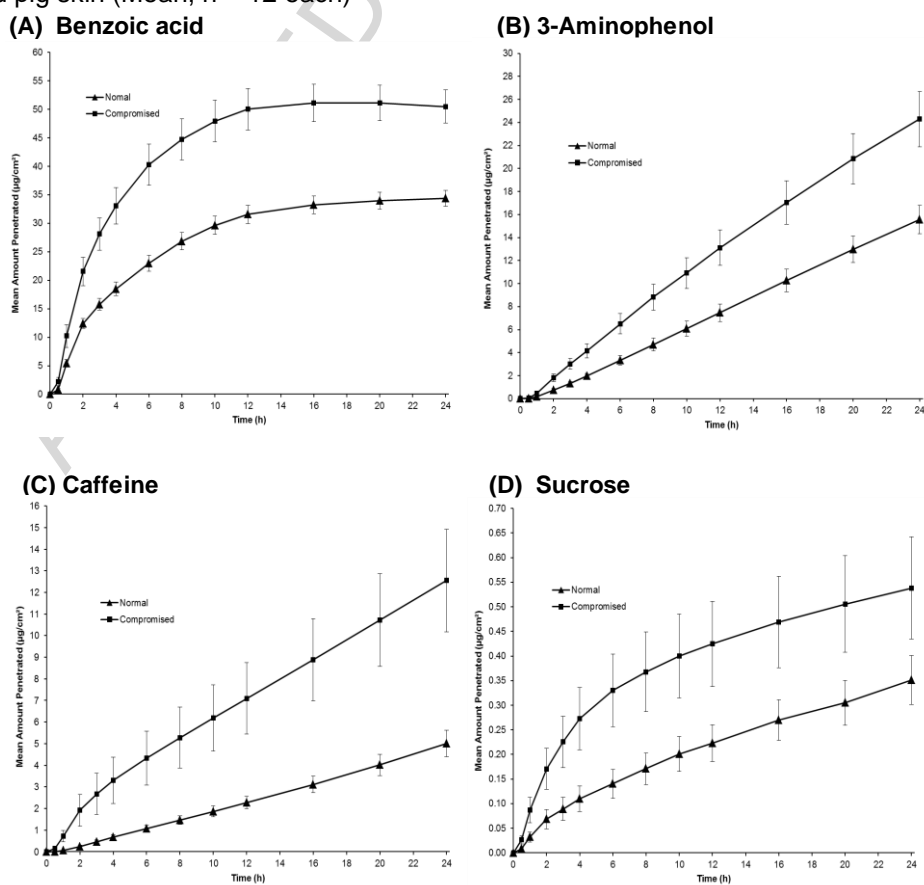
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Fig.1. Profile of skin penetration of reference chemicals through normal and compromised pig skin (Mean, n = 12 each)



Normal=Control untreated skin.
Compromised=10xTape stripped skin.

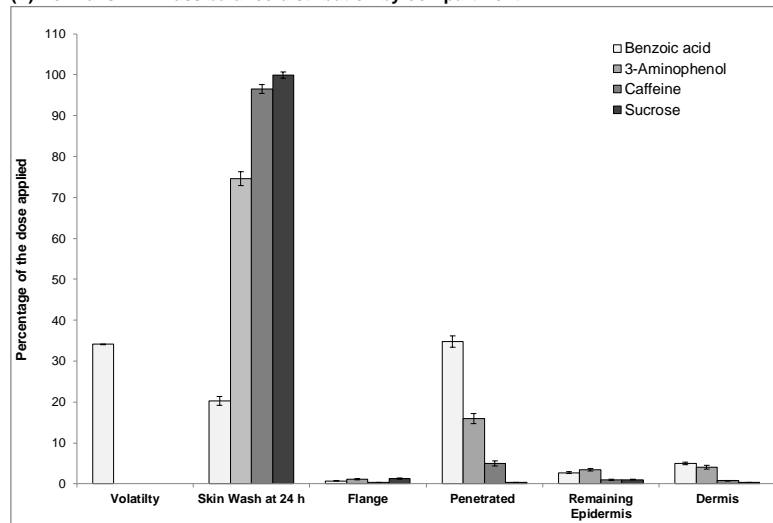
Fig.2 Individual comparison of the skin penetration of reference chemicals through normal and compromised pig skin (Mean, n = 12 each)



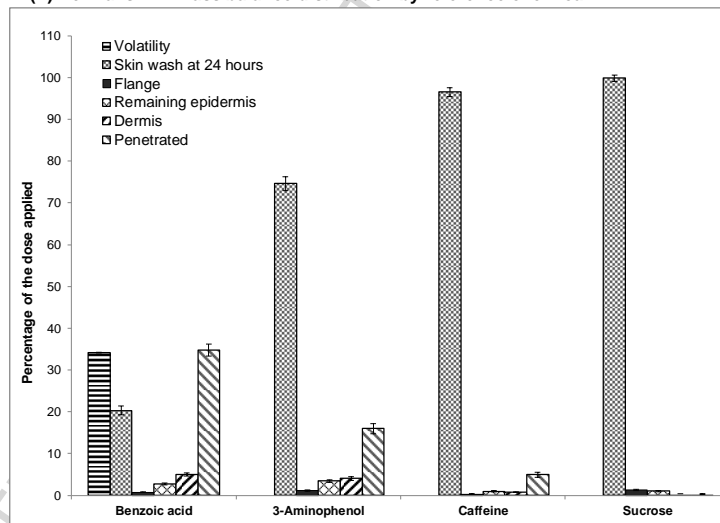
Normal=Control untreated skin.
Compromised=10xTape stripped skin.

Fig.3 Mass balance distribution of four reference chemicals through normal and compromised pig skin (Mean \pm SEM, n = 12 each)

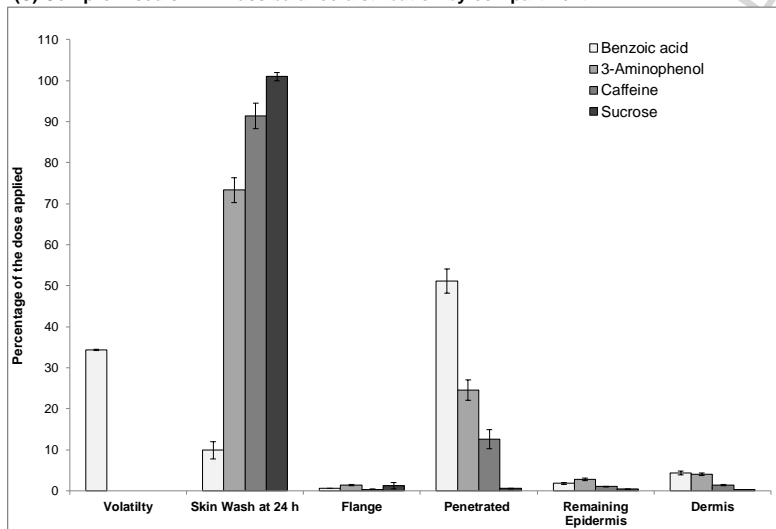
(A) Normal skin - Mass balance distribution by compartment



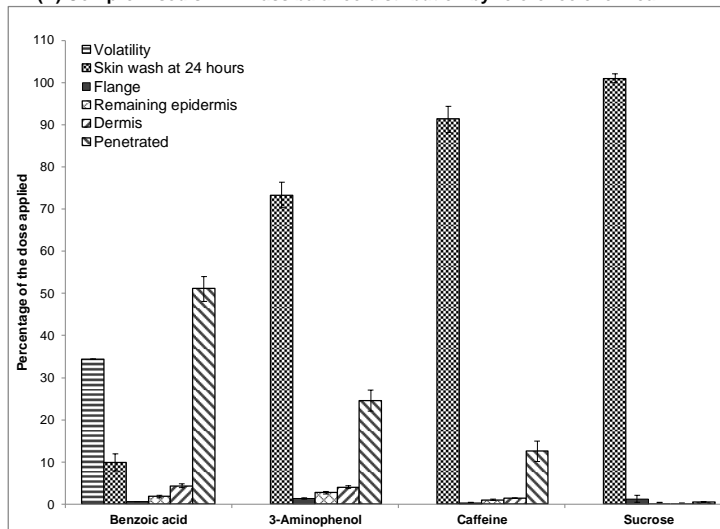
(B) Normal skin - Mass balance distribution by reference chemical



(C) Compromised skin - Mass balance distribution by compartment



(D) Compromised skin - Mass balance distribution by reference chemical



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Table 1

Physical and chemical properties of the reference chemicals tested

	Benzoic acid	3-Aminophenol	Caffeine	Sucrose
Molecular weight	122	109	194	342
Octanol-water partition coefficient (LogP _{ow})	1.87	0.18	-0.07	-3.76

Table 2

Absorption of Benzoic acid, 3-Aminophenol, Caffeine and Sucrose through normal and compromised pig skin (Mean \pm SEM, n = 12 each)

Amount ($\mu\text{g}/\text{cm}^2$)	Time (h)	Benzoic Acid		3-Aminophenol		Caffeine		Sucrose	
		Normal	Compromised	Normal	Compromised	Normal	Compromised	Normal	Compromised
	24	34.3 \pm 1.39	50.5 \pm 2.91	15.6 \pm 1.22	24.3 \pm 2.42	5.01 \pm 0.61	12.6 \pm 2.38	0.35 \pm 0.05	0.54 \pm 0.10
%	24	34.8 \pm 1.41	51.1 \pm 2.95	15.9 \pm 1.24	24.6 \pm 2.44	5.00 \pm 0.60	12.5 \pm 2.37	0.36 \pm 0.05	0.55 \pm 0.11
Rate ($\mu\text{g}/\text{cm}^2/\text{h}$)	0-24	1.42 \pm 0.06	1.99 \pm 0.11	0.67 \pm 0.06	1.05 \pm 0.11	0.21 \pm 0.03	0.51 \pm 0.10	0.01 \pm 0.002	0.02 \pm 0.004

Normal=Control untreated skin.

Compromised=10xTape stripped skin.

Table 3

Mass balance distribution through normal pig skin following 24 hour exposure to four reference chemicals (Mean \pm SEM, n = 12 each)

Compartment	Percentage dose applied			
	Benzoic acid	3-Aminophenol	Caffeine	Sucrose
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
Volatility	34.1 \pm 0.09	-	-	-
Donor Chamber	0.19 \pm 0.02	0.23 \pm 0.03	0.05 \pm 0.02	0.23 \pm 0.20
Skin Wash at 24 h	20.3 \pm 1.05	74.6 \pm 1.68	96.5 \pm 1.08	99.8 \pm 0.76
Flange	0.72 \pm 0.07	1.16 \pm 0.15	0.30 \pm 0.05	1.30 \pm 0.20
Remaining Epidermis	2.73 \pm 0.24	3.42 \pm 0.27	0.97 \pm 0.14	1.01 \pm 0.10
Dermis	4.99 \pm 0.33	4.05 \pm 0.46	0.76 \pm 0.05	0.36 \pm 0.02
Penetrated	34.8 \pm 1.41	15.9 \pm 1.24	5.00 \pm 0.60	0.36 \pm 0.05
Systemically available	42.5 \pm 1.20	23.4 \pm 1.57	6.73 \pm 0.70	1.72 \pm 0.15
TOTAL	97.8 \pm 0.93	99.4 \pm 0.50	104 \pm 0.86	103 \pm 0.49

Normal=Control untreated skin.

Systemically available=Penetrated+remaining

Table 4

Mass balance distribution through compromised pig skin following 24 hour exposure to four reference chemicals (Mean \pm SEM, n = 12 each)

Compartment	Percentage dose applied			
	Benzoic acid	3-Aminophenol	Caffeine	Sucrose
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
Volatility	34.4 \pm 0.12	-	-	-
Donor Chamber	0.25 \pm 0.06	0.49 \pm 0.11	0.16 \pm 0.05	0.063 \pm 0.02
Skin Wash at 24 h	9.88 \pm 2.06	73.3 \pm 3.05	91.3 \pm 3.10	101 \pm 1.03
Flange	0.61 \pm 0.05	1.33 \pm 0.17	0.36 \pm 0.06	1.27 \pm 0.76
Remaining Epidermis	1.81 \pm 0.25	2.76 \pm 0.28	0.99 \pm 0.13	0.40 \pm 0.06
Dermis	4.36 \pm 0.47	4.06 \pm 0.34	1.38 \pm 0.09	0.33 \pm 0.03
Penetrated	51.1 \pm 2.95	24.6 \pm 2.44	12.5 \pm 2.37	0.55 \pm 0.11
Systemically available	57.3 \pm 2.55	31.4 \pm 2.46	14.9 \pm 2.43	1.27 \pm 0.13
TOTAL	102 \pm 1.74	106 \pm 3.26	107 \pm 1.29	103 \pm 0.47

Compromised=10xTape stripped skin.

Systemically available=Penetrated+remaining

Highlights

- An *in vitro* model of pig skin compromised by tape stripping has been investigated
- Skin penetration and distribution of reference chemicals was established over 24h
- Although impaired, skin compromised using this model retained barrier capabilities
- This skin model could be useful for human risk assessment of consumer products

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