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

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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.

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, 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 (OECD, 2004a). 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 Nielsen, 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., 2012; Goon et al., 2004; Jakasa et al., 2006). However, these previous investigations were designed for specific scenarios and for specific chemical classes. Furthermore, previous

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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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, 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 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 accidently. 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 and Esdaile, 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 ± 1 °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 databridge and was expressed as kΩ/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, 1997; White et al., 2011). The chambers were allowed to equilibrate in a water bath at 32 °C for approximately 30 min after which electrodes were inserted into the 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 kΩ, 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 × 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/cm^2 for 5 s, 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 [^{14}C]-Benzoic acid, [^{14}C]-3-Aminophenol, [^{14}C]-Caffeine and [^{14}C]-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 doses (10 μL/cm^2) were applied to the surface of the skin membranes at a dose of 100 μg/cm^2 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 °C ± 1 °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

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