



Development of a Modified In vitro Skin Absorption Method to Study the Epidermal/Dermal Disposition of a Contact Allergen in Human Skin

Ruth U. Pendlington, Helen J. Minter, Leanne Stupart, Cameron MacKay, Clive S. Roper, David J. Sanders & Camilla K. Pease

To cite this article: Ruth U. Pendlington, Helen J. Minter, Leanne Stupart, Cameron MacKay, Clive S. Roper, David J. Sanders & Camilla K. Pease (2008) Development of a Modified In vitro Skin Absorption Method to Study the Epidermal/Dermal Disposition of a Contact Allergen in Human Skin, Cutaneous and Ocular Toxicology, 27:4, 283-294, DOI: [10.1080/15569520802327005](https://doi.org/10.1080/15569520802327005)

To link to this article: <http://dx.doi.org/10.1080/15569520802327005>



Published online: 02 Dec 2008.



Submit your article to this journal [↗](#)



Article views: 116



View related articles [↗](#)



Citing articles: 14 View citing articles [↗](#)

DEVELOPMENT OF A MODIFIED *IN VITRO* SKIN ABSORPTION METHOD TO STUDY THE EPIDERMAL/DERMAL DISPOSITION OF A CONTACT ALLERGEN IN HUMAN SKIN

Ruth U. Pendlington

Safety & Environmental Assurance Centre, Unilever Colworth, Sharnbrook, Bedford, UK

Helen J. Minter

Safety & Environmental Assurance Centre, Unilever Colworth, Sharnbrook, Bedford, UK

Leanne Stupart

In Vitro Sciences, Charles River Laboratories, Tranent, Edinburgh, UK

Cameron MacKay

Safety & Environmental Assurance Centre, Unilever Colworth, Sharnbrook, Bedford, UK

Clive S. Roper

In Vitro Sciences, Charles River Laboratories, Tranent, Edinburgh, UK

David J. Sanders

Safety & Environmental Assurance Centre, Unilever Colworth, Sharnbrook, Bedford, UK

Camilla K. Pease

Safety & Environmental Assurance Centre, Unilever Colworth, Sharnbrook, Bedford, UK

In vitro skin absorption methods exist in Organisation for Economic Co-operation and Development (OECD) guideline form (No. 428) and are used to estimate the degree of systemic penetration of chemicals through skin. More detailed kinetics of permeation through skin compartments are not described well by existing methods. This study was designed to assess the practical feasibility of generating compartmental (stratum corneum/epidermal/dermal) disposition and kinetic data of topically applied chemicals. For chemically induced effects initiated in the skin (e.g., skin allergy), the delivery of tissue concentrations of chemical will impact the incidence and severity of biological effect. Explicit data on the kinetics of chemical disposition in skin have not traditionally been needed for skin allergy risk assessment: current in vivo assays embody delivery implicitly. Under the 7th Amendment to the European Cosmetics Directive, in vivo assays (such as the local lymph node assay for skin sensitization) will not be permitted to assess cosmetic

Address correspondence to Dr. Camilla K. Pease, SEAC, Unilever, Colworth Science Park, Sharnbrook, Bedford MK44 1LQ, United Kingdom. E-mail: camilla.pease@unilever.com

ingredients. New in vitro and in silico alternative approaches and ways of predicting risk of adverse effects in humans need to be developed, and new methods such as that described here provide a way of estimating delivered concentrations and the effect of formulation changes on that delivery. As we continue to deconstruct the contributing factors of skin allergy in humans, it will be useful to have methods available that can measure skin tissue compartment exposure levels delivered from different exposure use scenarios. Here we provide such a method. The method could also be used to generate useful data for developing in silico kinetic models of compartmental skin delivery and for refining data for skin delivery in relation to the evaluation of systemic toxicity.

Keywords: Human skin penetration; Cinnamic aldehyde; *In vitro* alternatives; Skin allergen

INTRODUCTION

Drivers for new *in vitro* methods are emerging in the context of developing alternative non-animal methods to deliver new types of data for consumer safety risk assessment (1). The new legislation of the 7th Amendment to the European Cosmetics Directive (2) describes a marketing ban for ingredients that have been tested in *in vivo* assays, including tests for mutagenicity (beginning in March 2009) and tests for skin sensitization (beginning in 2013), for example, the murine local lymph node assay (LLNA) and guinea-pig maximization test (GPMT). To continue innovation with new chemicals, the cosmetics industry will have to develop new alternative risk-assessment approaches using new *in vitro* and *in silico* models/methods for data generation and interpretation, to be translated in the context of an understanding of the human biology and clinical epidemiology of such end effects as skin cancer and skin allergy. As such, a general conceptual approach has been described (3), and we have been evaluating the feasibility of the approach for application in the skin allergy area (1). The generation of epidermal disposition data is a part of this new human risk-assessment approach.

For chemically induced effects that are initiated in the skin, such as skin allergy, the degree of exposure and the delivered concentration to the target skin tissue have an impact on the incidence and severity of its causative effect in humans. Traditionally, explicit data on the kinetics of chemical disposition in skin and its compartments (stratum corneum [SC], epidermis, and dermis) have not been needed for hazard characterization and skin allergy risk assessment, and *in vivo* assays have included the kinetics of skin delivery implicitly in their design. It can be expected that the kinetics of delivery of test compounds via the mouse ear into the skin and systemic circulation in the context of LLNAs is very different from the context of delivery of chemicals to human skin. New non-animal, human-relevant approaches will rely on deconstructing the contributing mechanisms of human skin allergy, and data on human skin bio-availability and target tissue dosimetry of contact allergens have been identified as a new parameter type of possible relevance for input into new non-animal skin allergy prediction models (such as the simple scoring system described by Jowsey et al. (4). At a recent expert workshop run by the European Centre for the Validation of Alternative Methods (ECVAM) on the topic of “Skin Sensitisation and Epidermal Disposition” (5), it was also concluded that the delivery of the epidermal concentration (i.e., target tissue dose) of sensitizing chemical arising from specific exposure scenarios is expected to be a key parameter in new integrated weight-of-evidence modeling approaches to

predict skin sensitization potential in humans. The term *epidermal disposition* was defined at this workshop to describe the relevant input parameter for future alternative modeling approaches to predict sensitization potential. It was also concluded at this workshop that *in vitro* methods did not currently exist for generating such epidermal disposition data, hence the need to develop the new method described here.

In vitro skin absorption methods are used today to investigate the degree and kinetics of absorption of chemicals into and through skin to give an overall estimate of the dose that reaches the systemic circulation. Such data are used routinely in systemic toxicology risk assessments for dermally applied products, and appropriate methods for data generation are described in Organisation for Economic Co-operation and Development (OECD) Guideline 428 (6). Detailed kinetics—i.e., the rate of entry into and the rate of exit from the 3 main heterogeneous compartments of the skin (SC, epidermis, and dermis) and ultimately into the blood supply—are not covered well by guideline *in vitro* skin penetration methods. Hence, this lack of robust methodology has stimulated the development of a modified *in vitro* skin absorption method, to generate data on skin compartmental (i.e., SC, epidermal, and dermal) concentrations of chemical.

This study was designed to assess the practical feasibility of generating SC/epidermal/dermal disposition and kinetic data (i.e., the temporally varying absorption and distribution through the SC, epidermis, and dermis of topically applied chemicals). As a first step, the standard *in vitro* skin absorption method described by OECD Guideline 428 (6) was adapted to provide time course data for the distribution of a contact allergen, cinnamic aldehyde, dosed in 4 different vehicles that are currently used in *in vivo* skin sensitization protocols. An investigation of observed vehicle differences using the method was performed, as an example to illustrate how the method might be used to gain new insights as well as generate quantitative input data for risk assessment.

EXPERIMENTAL

Chemicals

Trans-[phenyl- U - ^{14}C]-cinnamic aldehyde was supplied by Selcia Limited (Ongar, Essex, UK). Trans-cinnamaldehyde, 1,2-propanediol (propylene glycol), olive oil (highly refined, low acidity), phosphate-buffered saline, newborn calf serum, penicillin-streptomycin solution, and amphotericin B were supplied by Sigma-Aldrich Company Limited (Gillingham, Kent, UK). Aquasafe 500 liquid scintillation fluid was obtained from Zinsser Analytic (Maidenhead, Berkshire, UK). Solvable was supplied by Perkin-Elmer LAS (UK) Limited, (Beaconsfield, Buckinghamshire, UK). All other general reagents were of the highest quality available. Simple antibacterial hand wash soap was supplied by Accantia Health and Beauty Limited (Birmingham, UK).

Skin Samples

Full-thickness human skin (2 abdominal and 2 breast samples) were obtained from female patients (aged 19 to 46 years old) who gave informed consent for their skin to be taken for scientific purposes prior to undergoing routine surgery. The skin was cleaned of subcutaneous fat and connective tissue using a scalpel blade. The skin

samples were then wrapped in aluminium foil, placed in sealed plastic bags, and stored at -20°C until used in the study. Human split-thickness skin (200–400 μm) was prepared from thawed skin samples using a Zimmer Ltd (Swindon, UK) electric dermatome.

METHOD

Skin samples from the same donors were used in each experiment, so that any differences seen with vehicle were not compounded by differences in donor. An “experiment” comprised 30 diffusion cells: in each experiment, 6 skin samples from each of donors 1, 2, and 3 and 12 skin samples from donor 4 were used (6 for mass balance purposes and 6 for microautoradiography at each time point). The skin from donors 1 and 3 was breast skin and that from donors 2 and 4 was abdominal skin. The skin samples were mounted in flow-through diffusion cells, which were set in a heated steel manifold to give a skin surface temperature of 32°C . Receptor solution—phosphate-buffered saline containing 5% newborn calf serum, amphotericin B (250 $\mu\text{g}/\text{ml}$), streptomycin (0.1 mg/ml), and penicillin G (100 U/ml)—was pumped through the cells for a 15-minute equilibration period before dosing.

One test preparation was administered per experiment. The test preparations were 25 mg/ml (2.5%, w/v, 189 mM) [^{14}C]-cinnamic aldehyde in each of the following vehicles:

- Propylene glycol
- Acetone:olive oil (4:1, v:v)
- Aqueous ethanol (50%, v:v)
- Absolute ethanol

A dose of 25 μl (containing approximately 1 μCi) was applied evenly over the SC surface of the exposed skin using an M25 Gilson (Anachem, Luton, UK) Microman positive displacement pipette. The surface area was 0.64 cm^2 to give a cinnamic aldehyde dose of $625\text{ }\mu\text{g}/\text{cm}^2$, $4.73\text{ }\mu\text{mol}/\text{cm}^2$. The donor chambers of the cells were occluded with an activated charcoal filter trap and cap to collect the volatile fraction. After dosing, receptor solution was collected in hourly fractions from each cell. At each terminal time point (0.5, 1, 2, 4, 8, and 24 hours), 1 cell from each skin donor (1–4) was taken for mass balance calculations and another cell from skin donor 4 was taken for microautoradiography.

At each terminal time point, the carbon traps were removed from the cell and extracted with methanol. An aliquot (50 μl) of concentrated commercial soap was applied to the exposed skin and cleansed with tissue paper in a gentle rubbing motion. The skin was then rinsed with ten 0.5-ml aliquots of simple antibacterial hand wash soap solution diluted with water (2% soap, v/v); the washing process was then repeated. The skin was then removed from the cells and the cells were soaked in methanol. The skin samples were tape stripped (i.e., layers of SC were removed using discs of tape) ten times (analyzed individually) to remove all of the SC. The stripped skin was separated into dosed area and flange (non-dosed) area and the epidermis/dermis of the dosed area was separated by heat treatment. The inner dosed area of skin was wrapped in cling film (plastic wrap). A 200-g brass weight, heated to 60°C , was applied for 90 seconds to the upper surface of the skin. The skin was then unwrapped and the epidermis was removed by scraping with forceps. Tape strips and each skin subfraction were solubilized in Solvable. Radioactivity was measured by

scintillation counting each receptor solution fraction, skin rinse, skin swab, cell rinse, tape strip, epidermis, dermis, flange skin, and carbon trap. The sample taken at each time point for microautoradiography was rinsed, swabbed dry, and then snap-frozen in liquid nitrogen and stored at -70°C until it could be processed.

Microautoradiography

Microscope slides were prepared by washing with 1% hydrochloric acid in 95% ethanol/water (v:v), then rinsing with distilled water. The slides were dipped in gelatin “subbing” solution (2.6 g gelatin, 0.5 g chromium potassium sulfate in 500 ml pure water) and dried at 37°C . The slides were dipped in Ilford K5 emulsion under safelight conditions and dried. Slides were stored at -20°C in a light-proof box until required.

The pieces of skin were mounted using OCT embedding matrix tissue freezing medium at -20°C and allowed to stabilize for 30 minutes. The pieces were trimmed and 8- μm sections were cut using a Leica Microsystems (Milton Keynes, UK) CM 3050 cryostat. The sections were applied to the emulsion-coated slides and placed in autoradiography boxes.

The microautoradiography slides were developed after 6 or 8 days of exposure. The emulsion was developed using Ilford Phenisol (diluted 1:4 [v:v] with pure water) for 2 minutes. The slides were transferred to a stop bath (1% [v:v] aqueous acetic acid) for 1 minute then fixed in Ilford (Cheshire, UK) Hypam (diluted 1:4 [v:v] with pure water) for 3 minutes. Finally, the slides were rinsed well in running tap water and fixed in formal saline, and the sections were stained with hematoxylin and eosin, dehydrated through a series of alcohols and mounted in DPX mountant.

Photomicroscopy was carried out using a Leica Microsystems (Milton Keynes, UK) DMRB microscope and a JVC KY-F75U camera in conjunction with an OASIS (Cambridge, UK) 2000 image archive system.

RESULTS

Skin Penetration—Flux Data

Figures 1 and 2 illustrate skin penetration flux data (as measured in receptor fluid) for cinnamic aldehyde, as delivered from the 4 vehicles as dosed at $4.73\ \mu\text{mol}/\text{cm}^2$. The vehicle could be seen to have a large effect on the kinetics of “systemic penetration” of the cinnamic aldehyde through human skin over a 24-hour period. Penetration of the cinnamic aldehyde into the receptor solution was most rapid from the aqueous ethanol application, followed by the ethanol solution (Fig. 1). The penetration from the propylene glycol, although much slower than that from ethanol, resulted in an equal total amount (area under the curve) of cinnamic aldehyde reaching the receptor solution by the end of the experiment, as seen in the cumulative flux curve (Fig. 2) and total penetrated over 24 hours (Table 1). Delivery through human skin from an acetone:olive oil vehicle was the slowest of the 4 and led to a 2-fold less “total amount penetrated” over 24 hours than with aqueous ethanol as a vehicle.

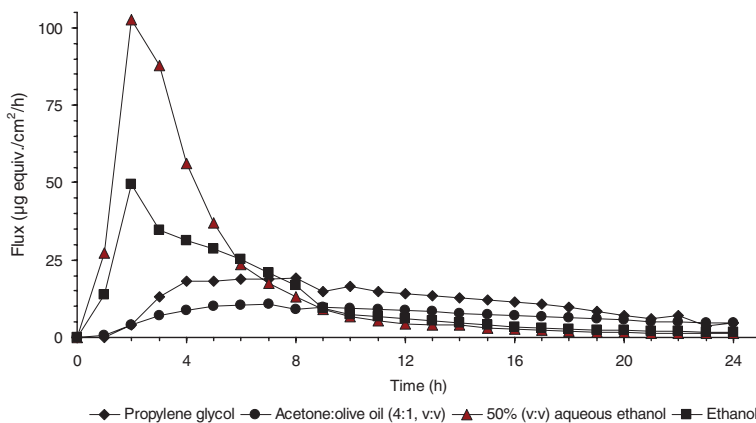


Figure 1 Flux of cinnamic aldehyde through human split-thickness skin from 4 different vehicles over 24 hours.

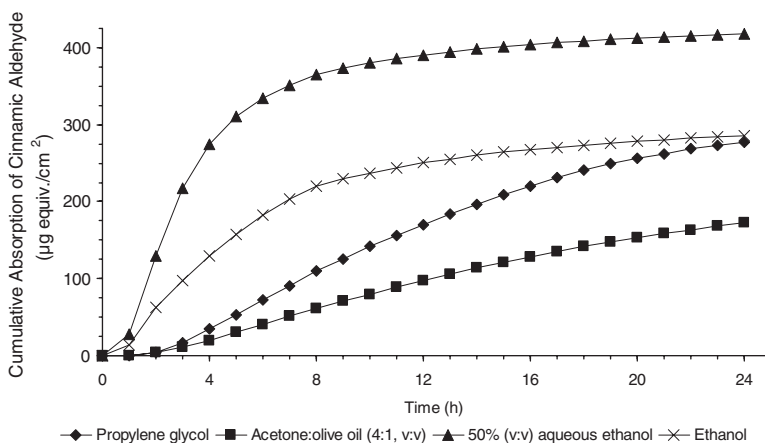


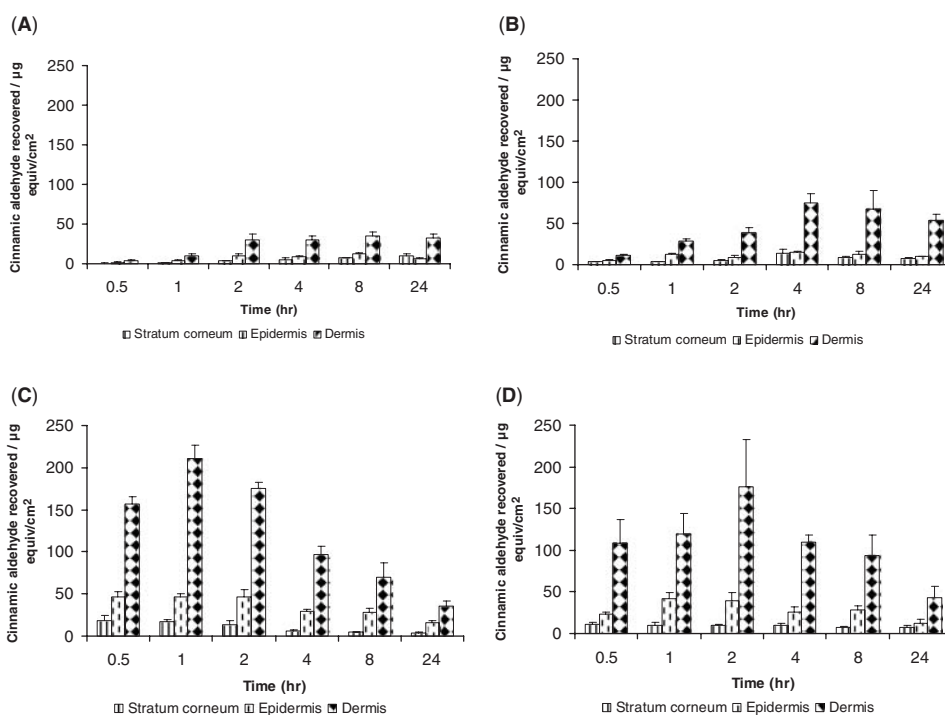
Figure 2 Cumulative penetration of cinnamic aldehyde through human split-thickness skin and into receptor fluid from 4 different vehicles over 24 hours.

Epidermal/Dermal Disposition

Distribution within the skin compartments was quite different, depending on the vehicle used and how data are interpreted (Figs. 3 and 4). Figure 3 compares the “ $\mu\text{g equivalent per cm}^2$ ” levels of chemical present in each of the SC/epidermal/dermal skin compartments as delivered from each vehicle. Comparatively low levels of cinnamic aldehyde were present in all skin compartments at all time points when dosed at $625 \mu\text{g/cm}^2$ (equivalent to $4.73 \mu\text{mol/cm}^2$) from either (A) propylene glycol or (B) acetone:olive oil. Levels in epidermis and dermis were higher using (C)

Table 1 Quantities of cinnamic aldehyde recovered from different compartments of the skin (A) as total amount and (B) as concentration

	Polyethylene glycol (<i>n</i> = 4)	Acetone:olive oil (4:1, v:v) (<i>n</i> = 4)	50% (v:v) aqueous ethanol (<i>n</i> = 4)	Ethanol (<i>n</i> = 4)
(A) Distribution of total amounts of cinnamic aldehyde in different skin fractions ($\mu\text{g equiv/cm}^2$) and total overall plus and minus stratum corneum (SC) amounts at termination of the experiment (24 hours) ^a				
Receptor fluid	279.59 (42.07)	174.42 (13.90)	417.97 (20.29)	286.15 (90.90)
Stratum corneum	10.35 (4.83)	7.97 (1.84)	4.11 (1.94)	7.91 (4.45)
Epidermis	6.56 (1.44)	9.61 (1.32)	15.52 (6.26)	12.32 (10.68)
Dermis	32.06 (12.06)	53.64 (15.50)	35.61 (12.33)	42.62 (28.97)
Total–SC	318.21 (32.38)	237.67 (18.94)	469.10 (8.33)	341.09 (110.24)
Total + SC	328.56 (31.95)	245.64 (20.31)	473.21 (9.96)	348.99 (111.57)
(B) Concentrations of cinnamic aldehyde in different skin compartments ($\mu\text{mol/cm}^3$) ^b				
Receptor fluid	—	—	—	—
Stratum corneum	76.88 (17.92)	59.22 (6.82)	30.53 (7.20)	58.73 (16.54)
Epidermis	6.97 (0.77)	10.20 (0.70)	16.47 (3.32)	13.07 (5.67)
Dermis	2.38 (0.45)	3.98 (0.55)	2.65 (0.46)	3.17 (1.08)

^aFigures in parentheses are the standard deviations.^bFigures in parentheses are the standard error of the mean.**Figure 3** Distribution of cinnamic aldehyde within the skin: (A) propylene glycol, (B) acetone:olive oil (4:1, v:v), (C) aqueous ethanol (50%, v/v), (D) ethanol expressed as $\mu\text{g equiv cinnamic aldehyde/cm}^2$. Error bars denote standard deviation.

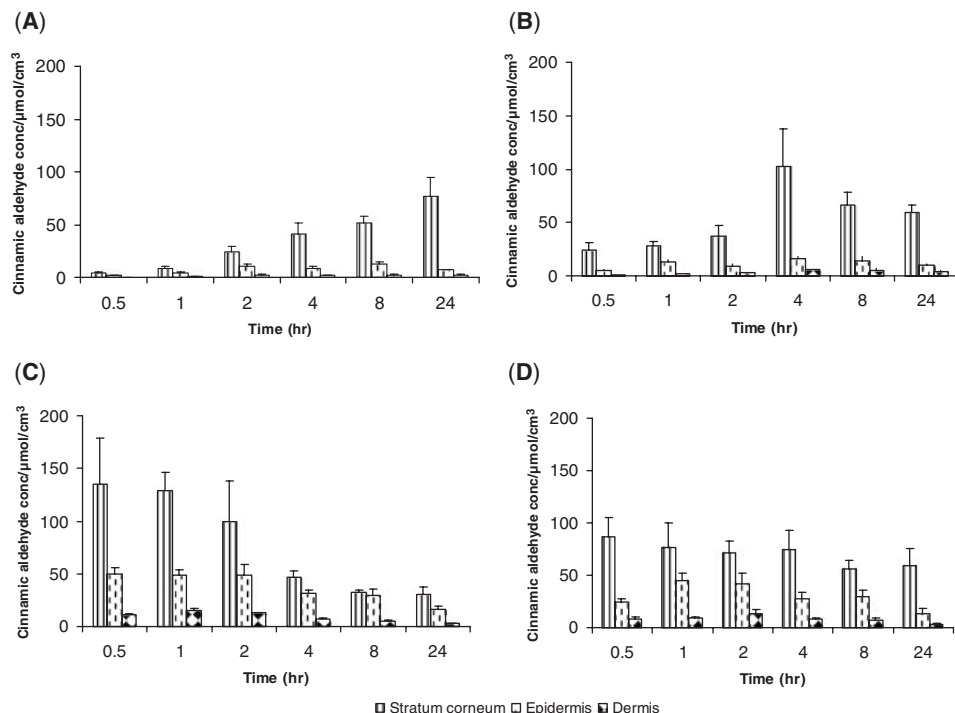


Figure 4 Distribution of cinnamic aldehyde within the skin: (A) propylene glycol, (B) acetone:olive oil (4:1, v:v), (C) aqueous ethanol (50%, v/v), (D) ethanol expressed as $\mu\text{mol}/\text{cm}^3$. Error bars denote standard deviation.

aqueous ethanol or (D) ethanol as vehicle. The highest amount of cinnamic aldehyde was always apparent in the dermis using the standard calculation of $\mu\text{g equiv}/\text{cm}^2$.

When the results are expressed as tissue concentrations mg/cm^3 or mol/cm^3 tissue, the pattern of distribution changes (Fig. 4). For these calculations, the depth of each layer was assumed to be: SC $12\ \mu\text{m}$, epidermis $70\ \mu\text{m}$, and dermis $1,000\ \mu\text{m}$.

Stratum Corneum Disposition

Distribution within the SC (as indicated by the analysis of individual tape strips) was also markedly different depending on the vehicle used (Fig. 5). In the case of the (A) propylene glycol and (B) acetone:olive oil vehicles, cinnamic aldehyde levels built up in the SC over time, indicating the formation of a reservoir. This is borne out by the flux data, which show that the flux of cinnamic aldehyde from these formulations effectively reached steady state. In the case of the (C) aqueous ethanol vehicle, cinnamic aldehyde moved into the SC rapidly, but levels dropped by the end of the experiment. With the (D) ethanol vehicle, levels in the SC were similar at all time points. Again, these results were compatible with the flux curves, which showed a rapid initial flux, followed by a decrease in flux as the remaining dose on the skin surface became rate limiting.

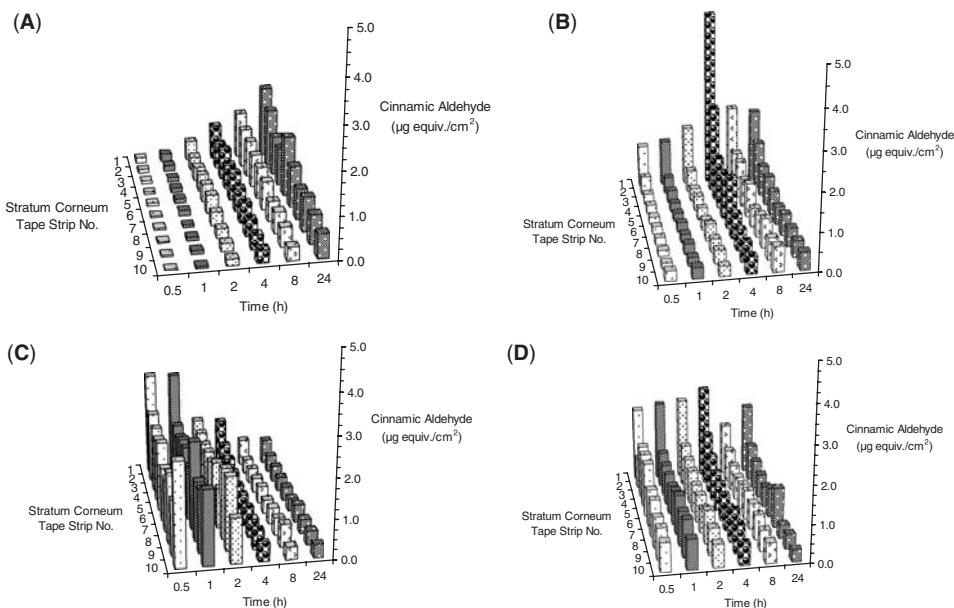


Figure 5 Distribution of cinnamic aldehyde within the stratum corneum after application in different vehicles: (A) propylene glycol, (B) acetone:olive oil (4:1, v:v), (C) aqueous ethanol (50% v:v), (D) ethanol. (Tape strip 1 is the first strip taken and therefore represents the outermost layer of the stratum corneum.)

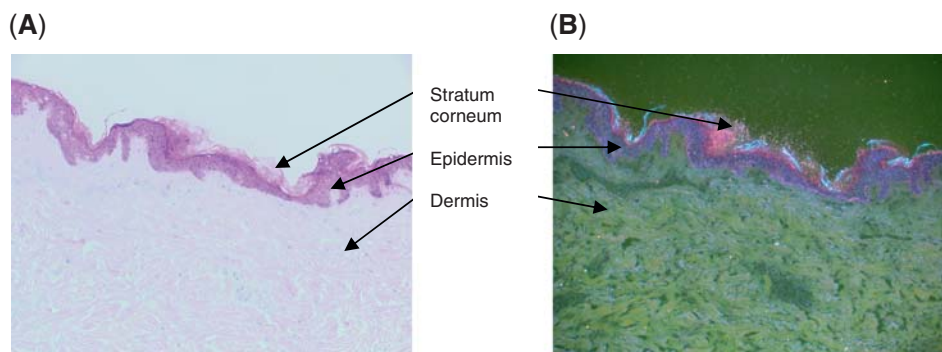


Figure 6 Distribution of cinnamic aldehyde within the skin after application in propylene glycol for 2 hours: (A) bright field image; (B) dark field image (silver grains indicating presence of radioactivity appear white).

Microautoradiography

This is a technique for visualizing a test item within the skin structure histologically. The microautoradiography results mirrored the mass balance results (Figs. 6 and 7, shown for the 2-hour time point for propylene glycol and 50% aqueous ethanol). Photomicroscopy was carried out under dark and bright field illumination. The location of radioactivity is visualized as white silver grain distribution under dark field

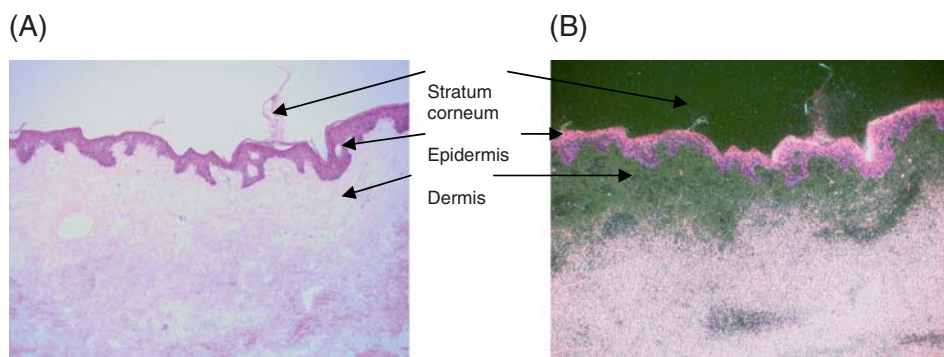


Figure 7 Distribution of cinnamic aldehyde within the skin after application in aqueous ethanol (50%, v/v) for 2 hours: (A) bright field image; (B) dark field image (silver grains indicating presence of radio-activity appear white).

illumination (background was defined as the level of silver grains seen on the untreated control sections [not shown] and on the emulsion adjacent to the sections). In the case of the propylene glycol vehicle, silver grains can be seen associated with the SC, whereas with the aqueous ethanol vehicle, the greatest concentration of silver grains can be seen in the dermis.

DISCUSSION

This pilot study has shown that the standard *in vitro* skin penetration method can be adapted to produce robust time course data on the SC/epidermal/dermal disposition of a contact allergen in human skin. The data from such a study design can be interpreted and used in a number of ways, e.g., deriving measurements of the area under the curve (AUC), maximum concentration (C_{max}), and maximal flux (J_{max}). This method can also be used to assess the impact of formulation on skin delivery to human epidermis. However, in the first instance it would be prudent to use this method for more sensitizing chemicals to assess its applicability.

The administered dose (600 µg/cm²) of the sensitizer cinnamic aldehyde was chosen to be comparable to that which is a sensitizing dose as revealed by LLNA data (775 µg/cm²) and human repeat insult patch test data (590 µg/cm²) (7). Previously, the skin absorption properties of cinnamic aldehyde have been investigated using a standard skin penetration method with neat chemical (78 µmol/cm²) (8), but quantification of levels in the skin compartments was not performed in this previous work because of the unavailability of radiolabeled chemical. Hence, this study and the previous work are not directly comparable in terms of quantification. The study presented here improves upon previous work and uses the more sensitive analytical technique of radio scintillation counting and with a much lower dose than in previous work, which is expected to better represent a finite dosing situation more akin to human exposure scenarios.

To date, skin absorption data have not been a required quantitative component of skin allergy risk assessments, because of the implicit nature in which skin

delivery is factored into *in vivo* assays for skin sensitization and because such risk assessments are often performed using benchmarked external dose only. However, uncertainty factors for vehicle delivery systems are factored in (9), but often without referral to actual skin delivery data. The magnitude of differences in skin delivery from the use of different vehicles will depend on the chemical. Distribution of cinnamic aldehyde in this study was different depending on the vehicle used under identical experimental conditions. The total amount of radiochemical absorbed by the skin (i.e., SC + epidermis + dermis + receptor fluid, as per Table 1) ranged from 245 (acetone:olive oil) to 473 (50% aqueous ethanol) $\mu\text{g equiv}/\text{cm}^2$. This would suggest that there can be up to a 2-fold difference in total delivery for cinnamic aldehyde between the different vehicles used. Assuming that these vehicles represent the best and worst vehicles for skin delivery, then uncertainty factors for vehicle differences could be modified in risk assessments based on such observed differences.

As we seek to find alternative non-animal methods to predict sensitization, we are deconstructing the chemico-biological mechanisms of contact allergy, in particular, to determine the key steps of the sensitization phase (1), and the dosimetry of delivery of chemical to human epidermis has been identified as one such parameter of potential relevance. The data here can be interpreted to estimate different parameter values (e.g., total amount in compartment expressed as $\mu\text{g equiv}/\text{cm}^2$, concentration mol/cm^3 tissue at selected time points, rate of penetration, maximal compartmental concentrations, diffusion-controlled rates of entry into and out of compartments, and penetrated, total concentrations delivered both to compartments and systemically at a given time point). It is not yet known which parameter derived from this methodology will be of ultimate relevance to skin allergy risk assessment, and more sophisticated data interpretation methods (using *in silico* kinetic modeling) and integration with other types of data will be the topic of further work to ascertain this. It should also be noted here that cutaneous metabolic conversion of cinnamic aldehyde, as seen to occur in human skin (8), is not taken into account in this study, as the skin (from frozen sources) was not considered metabolically active. Metabolism would be factored into the whole assessment as another piece of input information, generated using metabolism assays.

This paper serves to illustrate the type of study design that is anticipated to be most useful for generation of kinetic data types most relevant to integrated modeling and skin allergy risk assessment. For example, this method could provide useful information to ascertain whether equidosing in human skin has been affected from different chemicals in different exposure scenarios, e.g., with different vehicles. Already it can be seen from this work that vehicle can have a direct and significant impact on human skin epidermal disposition.

However, it is important to stress that these data are not considered in isolation with respect to correlating to sensitization potential, but that they will now be taken in context and with other appropriate data (e.g., from protein haptenation assays or *in vitro* cell-based assays) in an integrated manner to interpret and translate appropriate conclusions. Our objective is to develop tools and approaches to integrate data of diverse types to facilitate their interpretation for consumer safety risk assessment (1). We are working to develop and evaluate 3 different kinds of approaches: (a) "weight of evidence" methods, including simple scoring, and more complex statistical and Bayesian approaches; (b) *in silico* mechanistic modeling

(e.g., the Entelos Inc. Skin Sensitisation Induction Physiolab Platform (SSIPP) (1); and (c) biological network mapping and analysis of the modulation of human molecular interactions by chemicals. It is foreseeable that these data could be taken into quantitative human risk-assessment approaches for skin allergy, within a future new framework, and such application will also be the topic of further work (1).

Data derived from this experimental method will also be useful in the longer term to the *in silico* kinetic modeling community. In particular, works akin to those of Bunge and colleagues and Kasting and colleagues (as reviewed in 7), would benefit from having empirical data on a set of chemicals using the methods described here. *In vitro* experimental methods to generate quantitative data are laborious and expensive (especially when using radiochemicals, which we consider to be important for quantitative work). It is envisaged that robust *in silico* models of skin delivery and SC/epidermal/dermal concentrations could be developed for use in the future to provide human skin exposure estimates for finite dosing scenarios, in a quicker, cheaper, higher throughput way than can be done *in vitro*. However, *in silico* modeling should be built on a good set of empirical data of adequate chemical coverage. We have developed a method that can be used to generate such data for the purposes of both *in silico* kinetic modeling and improving risk assessment.

REFERENCES

1. Fentem J, Carmichael P, Maxwell G, Pease CK, Reynolds F, Warner G, Westmoreland C. Exploring new approaches to assess animal safety without animal testing, Proceedings of the World Congress on Alternatives Japan, 2008.
2. EU Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. Official Journal of the European Union 2003; L66:26–35.
3. Fentem J, Chamberlain M, Sangster B. The feasibility of replacing animal testing for assessing consumer safety: a suggested future direction. Alternatives to Laboratory Animals (ATLA) 2004; 32:617–623.
4. Jowsey IR, Basketter DA, Westmoreland C, Kimber I. A future approach to measuring relative skin sensitising potency: a proposal. J Appl Toxicol 2006; 26:341–350.
5. Basketter D, Pease C, Kasting G, Kimber I, Casati S, Cronin M, Diembeck W, Gerberick F, Hadgraft J, Hartung T, Marty JP, Nikolaidis E, Patlewicz G, Roberts DW, Roggen E, Rovida C, van de Sandt J. Skin sensitisation and epidermal disposition: the relevance of epidermal disposition for sensitisation hazard identification and risk assessment. Alternatives to Laboratory Animals (ATLA) 2007; 35:137–154.
6. Organisation for Economic Co-operation and Development. Guideline for testing chemicals, Guideline 428: Skin absorption: *in vitro* method; Paris, France: OECD. 2004.
7. Basketter DA, Clapp C, Jefferies D, Safford B, Ryan CA, Gerberick F, Dearman RJ, Kimber I. Predictive identification of human skin sensitisation thresholds. Contact Dermatitis 2005; 53:260–267.
8. Smith CK, Moore C, Elahi E, Smart A, Hotchkiss SAM. Human skin absorption and metabolism of the contact allergens cinnamic aldehyde and cinnamic alcohol. Toxicol Appl Pharmacol 2000; 168:189–199.
9. Felter S, Robinson M, Basketter DA, Gerberick F. A review of the scientific basis of uncertainty factors for use in quantitative risk assessment for the induction of allergic contact dermatitis. Contact Dermatitis 2002; 47:257–266.