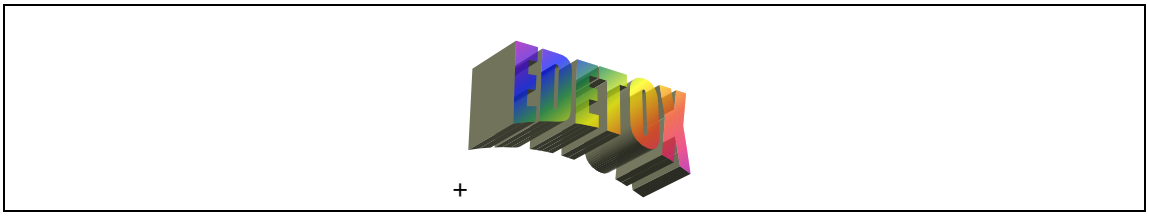

EUROPEAN COMMISSION

“Quality of Life and Management of Living Resources”

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Full Title

EVALUATIONS AND PREDICTIONS OF DERMAL ABSORPTION OF TOXIC CHEMICALS

Acronym

EDETOX

Final report for dissemination

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Contents

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Summary

This three year project aimed to generate new data on dermal absorption of chemicals. The project aimed to produce new knowledge that would (i) standardise *in vitro* systems for predicting percutaneous penetration and (ii) compare these to relevant *in vivo* studies. (iii) to use the in vitro system to generate occupationally relevant dermal absorption data acceptable for risk assessment and (iv) to evaluate predictive models of skin penetration of health related chemicals. A databank of carefully evaluated literature data has been produced and is available on the web http://edetox.ncl.ac.uk and these data and those generated during the project have been used in the QSAR and predictive models. The programme was composed of five work packages.

WP1 involved 10 members of the consortium assessing the robustness of the in vitro approach to prediction percutaneous penetration of chemicals. A protocol was developed in line with OECD Guidelines, agreed between participants, and inter-laboratory comparison of absorption of three marker chemicals caffeine, testosterone and benzoic acid was conducted. A paper has recently been accepted by Regulatory Toxicology and Pharmacology (Sandt et al 2004). In the robustness studies nine laboratories used human skin and one rat skin. Several further studies were stimulated by the results of WP1 to further define the influence of factors such as skin thickness, vehicle, receptor fluid that can influence derived diffusion parameters for marker chemicals. The penetration of 50% butoxyethanol/water was determined by 8 participants for comparison with parallel investigations of butoxyethanol dermal penetration by human volunteers in vivo.

The results from the studies in WP1 influenced the design of WP3 which aimed to obtain robust in vitro data on chemicals in occupationally relevant situations and to generate new data to test existing predictive models (WP4).

Parallel studies of percutaneous penetration through skin in human volunteers are essential to confirm the predictions of in vitro systems. Studies at AMC Amsterdam in WP2 concentrated on butoxyethanol (90% and 50% in water) as well as trichloroethylene and xylene vapour. The data was analysed using the mathematical deconvolution method. The design of these studies was directly in parallel with in vitro studies using human skin to evaluate the predictions from in vitro studies. Directly comparative in vitro and in vivo studies in humans and in rat were conducted. The results presented here add to the database used by organisations such as the OECD to confirm the usefulness of the in vitro approach The usefulness of prediction from in vitro studies was demonstrated when the influence of

aqueous vehicle on penetration of butoxyethanol was defined prior to designing the volunteer study. Parallel studies in which dermal microdialysis and urinary metabolite excretion were determined following application of 50% butoxyethanol in water were conducted between AMC and Erlangen. The microdialysis fluid only contained a small percentage of the absorbed material but interestingly preliminary analysis indicated some oxidative metabolism of butoxyethanol locally in the skin. Erlangen also conducted microdialysis studies of dermal absorption of pyrene in volunteers and these were compared with in vitro studies. Parallel in vivo/in vitro studies with butoxyethanol at a range of concentrations in water pyrene, benzopyrene and diethylhexylphthalate were conducted in the rat at INRS.

WP3 which aimed to generate occupationally relevant absorption data and data for QSAR was initiated after WP1. Guidelines on the design of in vitro studies based on the robustness study were circulated to participants. Finite dose data was generated for chemicals at occupationally relevant exposures, New data included **e**ffect of sweat on absorption of metal, effect of water on butoxyethanol dermal absorption, effect of vehicle on caffeine and pesticide absorption**,** dermal absorption data for an extended series of glycol ethers, natural oils, pesticides and absorption and metabolism of aromatic amines

The aim of WP4 was to evaluate existing predictive models of dermal absorption. This work package was therefore heavily dependent for some of its outcome on data generated in WP3. Details of the requirements for new data for inclusion in model assessment were included in the guidelines circulated to WP3. The aim was to generate infinite dose data that would allow the modellers to work with a parallel data base to that gathered by Flynn or by Cronin as the basis of most predictive models used today and to parallel this with absorption data t dose which were relevant to actual exposure. A databank of carefully evaluated literature data has been produced and is available on the web http://edetox.ncl.ac.uk. Data from the project will be added as it is published.

Comparisons of Kp derived from QSAR based on infinite doses of saturated aqueous solutions with in vitro determination of absorption for finite aqueous doses and doses in occupationally relevant vehicles indicated the limitations of using the QSAR approach for predicting absorption for risk assessment. Studies in the project revealed the influence of vehicle and formulation on the observed flux. and is a need to derive QSAR models that take into account vehicle effects.

A mechanistically-based mathematical model was developed during the project and that was used to interpret some of the data measured. This model enables the time courses of various variables to be simulated. modelling of a variety of exposure regimes, such as single and multiple dosing from different application forms can be handled. The development of these models and their testing through application to data measured in the EDETOX project has

resulted in considerable progress towards the development of a more reliable predictive tool for the estimation of the extent of dermal penetration of a chemical based on its physicochemical properties.

Background

Without acceptance of dermal absorption data generated mainly by in vitro approaches, risk assessment has been based on route to route extrapolation or default assumptions which in the worst case scenario have been 100 % dermal absorption even for chemicals which do not penetrate the skin well.

Reproducible absorption data is required to predict the risk of exposure to chemicals in a number of areas such as chemicals in the work place , agrochemicals, household products and cosmetics.

The OECD have established Guidelines and Guidance for the conduct of dermal absorption studies both in vivo and in vitro as have other organisations (EEC 1991, EC 2002, SCCNFP 2000). However consideration of the existing published literature indicated that there were insufficient parallel in vivo and in vitro studies in humans where the same dose protocol was applied to confirm the reliability of the in vitro approach. The EU under its Environment and Health Programme required further studies to influence decision making on acceptance of in vitro methods particularly within the framework of the 3Rs in studies of cosmetics. The proposed implementation of REACH has considerable impact on decisions in this area for dermal absorption of chemicals. Within this framework the EU funded the three year EDETOX Research Project.

OBJECTIVES (taken from original proposal)

The major objectives of the study were:

to investigate, in a group of centres in Europe, the usefulness of *in vitro* models to deliver relevant data on percutaneous penetration of chemicals. The reliability and interlaboratory variation will be assessed in order to establish standards for acceptability.

to demonstrate the relevance and predictability of these in vitro methods by conducting parallel in vivo measurements in human volunteers, and comparing in vitro and in vivo

data. Also, to conduct a limited comparative validation study with an animal model in order to provide a basis for interpretation and evaluation of literature-based animal data.

to generate data that will improve knowledge of the dermal absorption process for a series of important environmental and occupational contaminants which have health effects in man and for which percutaneous penetration may play an important role in the total internal exposure.

to use *in vitro* and *in vivo* experimental data generated in the project to evaluate and extend existing QSARs (quantitative structure activity relationships) that predict skin penetration and also evaluate and develop mathematical models of percutaneous penetrationand so that they can be used for the prediction of the rates of adsorption and the subsequent disposition of dermal penetrants.

overall to develop validated *in vitro* experimental strategies for the quantitative measurement of the dermal adsorption of chemicals and corresponding predictive computational models so that the use of animal testing will be greatly reduced in these risk assessment procedures.

to provide relevant quantitative data which can be used directly in the risk assessment for dermal exposure, including information that will allow regulatory authorities to progress towards assignment of quantitative skin notations for potentially hazardous chemicals.

to widely disseminate the results of this study to regulatory authorities and all other interested parties.

EXPECTED ACHIEVEMENTS

The objectives would lead to deliverables that would increase understanding of percutaneous penetration and generate guidelines and skin notations. The major deliverables would be:

A standardised method for in vitro studies of percutaneous penetration
In vitro/in vivo comparisons
In vitro data on percutaneous penetration of groups of chemicals for risk assessment
Evaluation and development of model systems
A data base of percutaneous penetration data

Work programme

The work programme was divided into five work packages each with a number of participants and deliverables. The work packages (WP) 1-4 ran in parallel and were inter-linked as indicated and WP5 prepared the final report. Participants contributed to several work packages.

For clarity methods and results from the 5 work packages are presented as separate sections

Work Package 1 Robustness of in vitro methodology

Work Package 2 In vivo measurements of dermal absorption

Work Package 3 In vitro measurements of dermal absorption

Work Package 4 Models and predictions of dermal absorption

Work Package 5 Dissemination of results and discussion of skin notation

Work Package 1- Robustness of in vitro methodology

Coordinator and report author J.J.M. van der Sandt, TNO

Objectives

WP1 was conducted as a preliminary to WP3 and had three objectives:

1) partially standardizing in vitro assays for in vitro skin absorption
2) establishing the robustness of the in vitro technology
3) generation of data on reference compounds for regulatory purposes
4) establishing in vitro assays for use in WP3 for generating occupationally relevant data

Summary

In order to obtain better insight into the robustness of *in vitro* percutaneous absorption methodology, the intra- and inter-laboratory variation in this type of study was investigated in 10 European laboratories. To this purpose, the *in vitro* absorption of 3 compounds through human skin (9 laboratories) and rat skin (1 laboratory) was determined. The test materials were benzoic acid, caffeine and testosterone, representing a range of different physico-chemical properties.

Methods

All laboratories performed their studies according to a detailed protocol in which all experimental details were described and each laboratory performed at least three independent experiments for each test chemical.

Results

All laboratories assigned the absorption of benzoic acid through human skin the highest ranking of the three compounds (overall mean maximum absorption rate of 16.54 ± 11.87 $\mu g/cm^2/h$). The absorption of caffeine and testosterone through human skin was similar, having overall mean maximum absorption rates of 2.24 ± 1.43 $\mu g/cm^2/h$ and 1.63 ± 1.94 $\mu g/cm^2/h$, respectively. In 7 out of 9 laboratories, the maximum absorption rates of caffeine were ranked higher than testosterone. No differences were observed between the mean absorption through human skin and the one rat study for benzoic acid and testosterone. For caffeine the maximum absorption rate and the total penetration through rat skin were clearly higher than the mean value for human skin. When evaluating all data, it appeared that no consistent relation existed between the diffusion cell type and the absorption of the test

compounds. Skin thickness only slightly influenced the absorption of benzoic acid and caffeine. In contrast, the maximum absorption rate of testosterone was clearly higher in the laboratories using thin, dermatomed skin membranes. Testosterone is the most lipophilic compound and showed also a higher presence in the skin membrane after 24 hour than the two other compounds. The results of this study indicate that the *in vitro* methodology for assessing skin absorption is relatively robust. A major effort was made to standardize the study performance, but, unlike in a formal validation study, not all variables were controlled. The variation observed may be largely attributed to human variability in dermal absorption and the skin source. For the most lipophilic compound, testosterone, skin thickness proved to be a critical variable. The results of the ring trial have been published in *Regulatory Toxicology and Pharmacology* [1]

In addition to benzoic acid, caffeine and testosterone, a fourth test compound was selected for inclusion in the ring trial: 2-butoxyethanol (50%, w/w). The study design for this compound was slightly different than for the other compounds (infinite dose of 200 µl/cm^2, 4-h exposure time under occlusion) in order to allow for a direct comparison with in vivo studies (volunteers, rats) performed in Work package 2. Each laboratory performed 3 experiments (table 1). The mean maximal flux was through human skin membranes was 1.5 ± 1.0 mg/cm^2/h (8 laboratories). The maximum absorption rate of 2-BE (50%) was clearly higher in the laboratories using thin, dermatomed skin membranes (2.8, 2.8 and 1.7 mg/cm^2/h) than in laboratories using non-dermatomed skin (0.5, 0.6, 0.7, 1.0 and 1.2 mg/cm^2/h). Through rat skin (1 laboratory), the maximum absorption rate of 2-BE (50%) was 1.6 mg/cm^2/h.

Table 1: In vitro percutaneous absorption of 2-BE (200 µl/cm^2, 4-h exposure time)

[1] van der Sandt, JJM, Maas, WJM, van Burgsteden, JA, Sartorelli, P, Montomoli, L, Larese, F Payan, J-P, Limaset, JC, Carmichael, P, Kenyon, S, Robinson, E, Dick, I , Nilsen Jb, Schaller K-H, Korinth, G, Cage, S Wilkinson, SC and Williams Faith M. In vitro prediction of skin absorption of caffeine, testosterone and benzoic acid : multicentre comparison study. *Regulatory Pharmacology and Toxicology 39 271-281* 2004

Participant No.	Experiment No.	Number of replicates	Species	Maximal Absorption Rate (mg/cm2/h)
1 (UNEW)	1 2 3 *mean +/- SD*	7 7 7	Human Human Human	1.8 4.4 2.1 *2.8 +/- 1.4*
2 (Siena)	1 2 3 *mean +/- SD*	ND	ND	ND
3 (Trieste)	1 2 3 *mean +/- SD*	7 7 7	Human Human Human	0.6 0.5 0.5 *0.5 +/- 0.1*
4 (TNO)	1 2 3 *mean +/- SD*	7 7 7	Human Human Human	1.1 1 1.2 *1.0 +/- 0.1*
5 (INRS)	1 2 3 *mean +/- SD*	8 8 8	Rat Rat Rat	1.4 1.9 1.5 *1.6 +/- 0.4*
6 (Imperial)	1 2 3 *mean +/- SD*	7 7 7	Human Human Human	1.1 1.5 1.1 *1.2 +/- 0.2*
7 (HSL)	1 2 3 *mean +/- SD*	5 5 5	Human Human Human	2.3 3.8 2.3 *2.8 +/- 0.9*
8 (Odense)	1 2 3 *mean +/- SD*	7 7 7	Human Human Human	0.6 0.6 0.5 *0.6 +/- 0.1*
9 (Erlangen)	1 2 3 *mean +/- SD*	7 7 7	Human Human Human	0.8 0.8 0.5 *0.7 +/- 0.2*
10 (HLS)	1 2 3 *mean +/- SD*	6 5 7	Human Human Human	1.4 1.8 1.8 *1.7 +/- 0.2*

Table 1: In vitro percutaneous absorption of 2-BE (50% v/v in water, 200 µl/cm^2, 4-h exposure time, except Erlangen [8 h exposure time]))

Influence of skin thickness

In a separate series of studies, the influence of skin thickness was investigated in further detail. Percutaneous penetration of caffeine, testosterone, propoxur (all at finite dose [100-137 µg/cm^2] in ethanol:water vehicle) and butoxyethanol (undiluted finite dose [25 µl/cm^2] or as a 50% [v/v] aqueous solution) through skin of varying thicknesses was measured using flow through cells for 8 to 24 h. Saline (pH 7.4) was used as receptor fluid, with 2% (w/v) BSA added for studies with testosterone and 3% (w/v) BSA added for studies with propoxur. Penetration of propoxur was tested under occluded conditions. Following exposure, the

remaining surface dose was removed by swabbing and the skin digested prior to scintillation counting. There was little influence of skin thickness on maximum flux or distribution of caffeine. Maximum flux and cumulative dose absorbed of testosterone and butoxyethanol (in both finite and infinite doses) were markedly reduced with full thickness (about 1 mm thick) skin compared with split thickness skin (about 0.5 mm). The presence of these test compounds in the membrane was not significantly affected by skin thickness. Maximum flux of propoxur exhibited a strong inverse correlation with skin thickness (0.5 – 1.3 mm), and retention of propoxur by the membrane increased significantly with skin thickness. The proportion of the dose absorbed after 24 h was also inversely correlated with skin thickness. Significant differences in retention and cumulative absorption of propoxur were measured between skin preparations 0.5 mm and 0.7 mm thick, whilst maximum flux was not significantly different between these thicknesses. From this series of experiments, it was concluded that a complex relationship exists between skin thickness, lipophilicity and percutaneous penetration and distribution. This has implications for risk assessment studies and for the use of data from different sources using skin samples of different thicknesses (a paper is in preparation[2])

Overall conclusions on the design of in vitro methods following the robustness study

The following important variables that should be controlled:

1. Variability between the absorption properties of samples of skin from different individuals was confirmed. Therefore at least three samples of skin are recommended and if there is an outlier up to six.
2. We recommend that skin be dermatomed to 300um for use in the in vitro cell.

Less important variables:

1 Choice of cell type

2 In this study inter-laboratory variability was less than inter-skin variability. However by comparison the study with artificial membrane (Chilcott et al 2005)[3] indicated the potential for significant variation in measurements of methylparaben absorption.

The study has reinforced the advice and guidance form the OECD on the conduct of in vitro absorption studies.

[2] Simon C. Wilkinson, Wilfred J.M. Maas, Jesper Bo Nielsen, Laura C. Greaves, Johannes J.M. van de Sandt, Faith M. Williams Interactions of skin thickness and physicochemical properties of test compounds in percutaneous penetration studies (2005 submitted Int Arch Env Hlth)

[3] Chilcott et al (2005) J Pharm Sci in press

Regulatory Pharmacology and Toxicology 39, 271-281 2004-

In vitro predictions of skin absorption of caffeine, testosterone and benzoic acid: a multi-centre comparison study

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Running title: Multi-centre skin absorption assessment

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Abstract

In order to obtain better insight into the robustness of *in vitro* percutaneous absorption methodology, the intra- and interlaboratory variation in this type of study was investigated in 10 European laboratories. To this purpose, the *in vitro* absorption of 3 compounds through human skin (9 laboratories) and rat skin (1 laboratory) was determined. The test materials were benzoic acid, caffeine and testosterone, representing a range of different physico-chemical properties. All laboratories performed their studies according to a detailed protocol in which all experimental details were described and each laboratory performed at least three independent experiments for each test chemical.

All laboratories assigned the absorption of benzoic acid through human skin the highest ranking of the three compounds (overall mean flux of 16.54 □g/cm^2/h). The absorption of caffeine and testosterone through human skin was similar, having overall mean maximum absorption rates of 2.24 □g/cm^2/h and 1.63 □g/cm^2/h, respectively. In 7 out of 9 laboratories, the maximum absorption rates of caffeine were ranked higher than testosterone. No

differences were observed between the mean absorption through human skin and the one rat study for benzoic acid and testosterone. For caffeine the maximum absorption rate and the total penetration through rat skin were clearly higher than the mean value for human skin. When evaluating all data, it appeared that no consistent relation existed between the diffusion cell type and the absorption of the test compounds. Skin thickness only slightly influenced the absorption of benzoic acid and caffeine. In contrast, the maximum absorption rate of testosterone was clearly higher in the laboratories using thin, dermatomed skin membranes. Testosterone is the most lipophilic compound and showed also a higher presence in the skin membrane after 24 hour than the two other compounds.

The results of this study indicate that the *in vitro* methodology for assessing skin absorption is relatively robust. A major effort was made to standardize the study performance, but, unlike in a formal validation study, not all variables were controlled. The variation observed may be largely attributed to human variability in dermal absorption and the skin source. For the most lipophilic compound, testosterone, skin thickness proved to be a critical variable.

Introduction

Reproducible data on percutaneous absorption in humans are required to predict the systemic risk from dermal exposure to chemicals, such as hazardous substances at the workplace, agrochemicals and cosmetic ingredients (EEC, 1991; EC, 2002; SCCNFP, 2000). In this context, there is a need for reliable *in vitro* models since the European Union advocates this approach and national legislation stipulates that animal experiments should be avoided whenever scientifically feasible. Furthermore, owing to the difference in skin structure, animal studies do not always reflect the human situation.

Absorption through the skin is the primary route of exposure for most pesticides both occupationally (Benford *et al*., 1999) and in residential settings (Ross *et al*., 1992). Despite the often relatively high dermal (and inhalation) exposure in occupational settings, regulations for pesticides and other chemical exposure have evolved from concern about the oral route of exposure. In the absence of reliable dermal absorption data, route-to-route extrapolation has been used to assess dermal risk. When no information is available on percutaneous absorption, risk assessments may assume an absorption percentage of 100%, a worst case scenario (EC, 2002). This is a very conservative approach and a more accurate measure of absorption would have a major impact on risk assessments for many chemicals in regulatory toxicology. The specific need for a valid method of assessing human dermal absorption has led the OECD (2000a,b,c) and EPA (1996, 1999) to produce guidelines for *in vitro* and *in vivo* assessment of percutaneous absorption.

A review of available data from published literature on *in vitro* dermal absorption was performed under the auspices of the OECD in order to evaluate the performance of *in vitro* and *in vivo* percutaneous absorption measurements. It was concluded that evaluation of *in vitro* test methods from published literature was difficult (OECD, 2000d) because studies containing direct comparisons of *in vitro* and *in vivo* measurements were very limited. There were too many variables, such as different species, thickness and types of the skin, exposure duration, vehicles. Also, very few multi-center studies have been performed (Beck *et al.*, 1994) and these studies were limited in their approach (*e.g*. with respect to the number of laboratories involved). Therefore, no proper data on the intra- and inter-laboratory reproducibility of the *in vitro* methodology are available.

The purpose of the present research was therefore to assess intra- and inter-laboratory variability in determination of percutaneous penetration by *in vitro* methods on a larger

scale than done previously. This report contains data generated by 10 independent laboratories from within the European Union, each testing the percutaneous absorption of 3 chemicals that are recommended by the OECD as suitable reference compounds for regulatory studies (OECD, 2000c). The experimental conditions (amount applied, exposure time, vehicle, receptor fluid, preparation of membranes, analysis) were standardized according to a detailed protocol that adopted many of the guidelines proposed by the OECD.

Materials and methods

Test substances and preparation of dose solutions

The test substances were chosen on the basis of their range in physico-chemical properties (Table 1), and their recommendation as reference compounds by the OECD (OECD, 2000c). All participating laboratories used the same batches of test substances. Non-radiolabeled testosterone, caffeine, benzoic acid were purchased from Steraloids Inc. (Newport R.I., USA) and Sigma Chemical Company by the study coordinator and were then supplied to the participants. [4-^{14}C]testosterone (53.6 mCi/mmol) and [1-methyl-^{14}C]caffeine (51.2 mCi/mmol) were purchased from PerkinElmer Life Sciences, while [ring-UL-^{14}C]benzoic acid (6.2 mCi/mmol) was obtained from Sigma Chemical Company. The dose solutions were prepared freshly by each laboratory in ethanol/water (1:1, v/v), yielding a concentration of 4.0 mg per mL for each compound. Participants with a license to handle radiochemicals prepared the dose solutions by mixing appropriate amounts of radiolabelled and non-radiolabelled test substances. The dose solutions were measured for exact total radioactivity prior to and directly after the application to the skin membranes. The radioactive concentration was approximately 1 MBq/mL for testosterone and caffeine and approximately 4 MBq/mL for benzoic acid.

Preparation of skin membranes

Both human and rat skin membranes were prepared from frozen skin. Whole skin was cleaned of subcutaneous fat and the skin was stored at approximately –20 ^{o}C (participants 1 and 2 at approximately –70 ^{o}C) for a maximum period of 1 year. The supply and use of human and animal tissue was in full accordance with national ethical guidelines. Detailed information on the human skin source was recorded (Table 2). Most participants used human skin with a thickness between 0.7 - 1.1 mm, while 3 laboratories used dermatomed skin with a thickness of 0.5 – 0.7 mm (participants 1 and 7) or 0.3 – 0.4 mm (participant 10). Skin from more than one donor was used in each experiment and each experimental group consisted of 5 to 7 skin membranes form different individuals. Rat full-thickness skin was used by participant 5 and was collected from the back (clipped carefully) of 4-weeks old male Sprague Dawley rats.

Diffusion cells and receptor fluid

Each participant used the diffusion cell that was established in their laboratory (details are shown in Table 3). For experiments with caffeine and benzoic acid, the receptor fluid consisted of saline (0.9 % NaCl), while for experiments with testosterone, the receptor fluid consisted of saline (0.9 % NaCl) + 5 % BSA, adjusted to pH 7.4. For systems using flow-through diffusion cells, the flow of receptor fluid was approximately 1.5 mL/h.

Experimental design

All participating laboratories performed their studies according to a detailed study protocol in which the experimental design and parameters such as the dose of the test chemical, vehicle, duration of the experiment, preparation of the skin membranes, receptor fluid type, occlusion, temperature, sampling times and number of replicates were defined. Skin membranes were thawed, mounted in the diffusion cell and the skin integrity was assessed by either visual assessment, permeation of tritiated water or capacitance, depending on the participant. Subsequently, the test substances were applied at a concentration of 4.0 mg per mL ethanol/water (1:1, v/v). The application volume was 25 μL/cm^2 which is considered the minimum volume necessary to produce a homogeneous distribution on the skin surface. This represented a finite dose (100 μg/cm^2), in order to mimic occupationally relevant situations. The exposure time was 24 h, during which the donor compartment remained occluded. Aliquots of the receptor fluid were collected at various time points (minimally at 1, 2, 4, 8 and 24 h post-dosing). For static cells, the original volume of the receptor fluid was restored by adding fresh receptor fluid to the receptor compartment directly after each sampling. In case of non-radiolabeled test compounds, the receptor fluid samples were stored at approximately –20 ^{o}C until analysis. At the end of the experiment, the test compound remaining at the application site was removed, using 5 cotton swabs dampened with ethanol/water (1:1, v/v), followed by 1 dry cotton swab. When a radioactive test compound was used, the cotton swabs, donor compartment rinse, receptor compartment rinse and skin membranes (after digestion with 1.5 M KOH in water/ethanol (1:4)) were analysed for presence of the test compound by □-counting. Each laboratory performed three to five independent experiments for each test chemical.

Analysis of non-radiolabeled test substances

The analysis of non-radiolabelled test substances in the dose solutions and receptor fluid samples was performed centrally: benzoic acid by the Health & Safety Laboratory (UK), caffeine by the University of Trieste (Italy) and testosterone by TNO Nutrition and Food Research (The Netherlands). Analysis for benzoic acid, caffeine and testosterone was performed by HPLC-UV according to established protocols. The amount of non-radiolabeled test substance was not determined in the skin tissue and therefore total recovery values were not calculated.

Analysis of radiolabeled test substances

Radioactivity measurements were made by individual participating laboratories. Radioactivity in the various samples (receptor fluid, skin, skin swabs and cell washings) was determined by liquid scintillation counting. Receptor fluid samples were added directly to an appropriate scintillation fluid. For analysis of the skin membranes, an aliquot of the tissue digest (1.5 M KOH in 20% aqueous ethanol) was used.

Calculation of results

The calculations were performed using a standardized Excel^{TM} spreadsheet prepared by the study coordinator. A cumulative amount absorbed per unit skin area versus time course was constructed from the amount of test substance in the receptor fluid and the maximum absorption rate was determined from the linear portion of the curve. The time to maximum rate, the percentage of the dose recovered in the receptor fluid in 24 hours, the percentage in the skin membrane and the percentage total recovery (for radiolabelled studies) was also calculated. The data of each laboratory were presented as mean ± standard deviation, together with the coefficient of variation (CV). The presence of the test compound in the skin membrane after washing the application area at 24 h was expressed by the ratio between the percentage of the dose in skin and receptor fluid (total penetration - TP) and the percentage of the dose in receptor fluid (RF).

Results

The absorption of caffeine, benzoic acid and testosterone through the skin was defined on the basis of maximum absorption rate, time to maximum rate, percentage dose recovered in the skin membrane (at 24 h post dosing), and percentage dose recovered in the receptor fluid (at 24 h post dosing). The results of individual laboratory measurements are shown in Tables 4 to 6 and overviews of the mean values are given in Figures 1 to 4.

Benzoic acid. The mean maximum absorption rate of benzoic acid through human skin membranes was 16.54 ± 11.87 □g/cm^2/h, while the amount in the receptor fluid after 24 h was 70.6 ± 17.2 % of the dose applied (8 laboratories). The mean maximum absorption rate of benzoic acid through rat skin (1 laboratory) was 21.21 □g/cm^2/h and the amount in the receptor fluid after 24 h was 89.8 %. For both human and rat skin, the ratio TP:RF was approximately 1.0, indicating that almost no benzoic acid remained in the skin membrane after washing the application area. The total recovery of the radioactivity ranged between 53.6 % and 98.5 % (7 laboratories).

Each laboratory performed 3 to 5 independent experiments. The coefficient of variation (CV) of the maximum absorption rate varied from 6.3 % (lab 4) to 52.2 % (lab 2). For the percentage in the receptor fluid (at 24 h), the CV values ranged between 1.6 % (lab 4) and 57.1 % (lab 2).

Caffeine. The mean maximum absorption rate of caffeine through human skin membranes was 2.24 ± 1.43 □g/cm^2/h, while the amount in the receptor fluid after 24 h was 24.5 ± 11.6 % of the dose applied (9 laboratories). The mean maximum absorption rate of caffeine through rat skin (1 laboratory) was 6.82 □g/cm^2/h and the amount in the receptor fluid after 24 h was 53.7 %. For both human and rat skin, the ratio TP:RF was only slightly higher than 1.0, indicating that only a small amount caffeine remained in the skin membrane after washing the application area. The total recovery of the radioactivity ranged between 66.4 % and 100.6 % (7 laboratories).

Each laboratory performed 3 to 5 independent experiments. The CV value of the maximaumabsorption rate varied from 12.0 % (lab 5) to 91.4 % (lab 1). For the percentage in the receptor fluid (at 24 h), the CV values ranged between 5.4 % (lab 5) and 66.0 % (lab 1).

Testosterone. The mean maximum absorption rate of testosterone through human skin was 1.63 ± 1.94 □g/cm^2/h, while the amount in the receptor fluid after 24 h was 11.8 ± 10.9 % of the dose applied (9 laboratories). The mean maximum absorption rate of testosterone through rat skin (1 laboratory) was 1.84 □g/cm^2/h and the amount in the receptor fluid after 24 h was 21.4 %. For both human and rat skin, the ratio TP:RF ranged between 1.35 and 3.54, indicating that a considerable amount testosterone remained in the skin membrane after washing the application area. The total recovery of the radioactivity ranged between 52.3 % and 103.5 % (7 laboratories).

Each laboratory performed 3 to 5 independent experiments. The CV value of the maximum absorption rate ranged from 6.3 % (lab 7) to 111.0 % (lab 8). For the percentage in the receptor fluid (at 24 h), the CV values ranged between 12.6 % (lab 7) and 111.7 % (lab 8).

Discussion

The presence of international guidelines has led to a partial standardization of *in vitro* skin absorption studies for regulatory purposes. On the other hand, the guidelines allow for certain flexibility in order to study compounds with widely differing physicochemical properties and under circumstances which are the most relevant for its use, resulting in *e.g*. different exposure times, dose levels and vehicle/formulations. In the OECD guidance document (OECD, 2000c), useful information is provided on how to properly design *in vitro* and *in vivo* skin absorption studies. Both static and flow-through diffusion cell types are considered suitable. In order to prevent underestimation of skin absorption, the test compound should be soluble in the receptor fluid, but the receptor fluid should not alter the barrier properties of the skin membrane. Skin membranes can be prepared in various ways, but the use of skin membranes with a thickness of more than 1.0 mm (epidermis and dermis) is not recommended and must be justified by the researcher, since the absorption of lipophilic compounds may be impeded by a thick dermis. This guidance has been proved useful for both investigators in the laboratory and for regulatory agencies which evaluate this type of data for risk assessment purposes.

Only very limited data exist on the intra-laboratory and inter-laboratory variation of *in vitro* skin absorption studies. In 1994, Beck *et al*. reported a good correlation of *in vitro* absorption of hair dyes through full-thickness pig skin in 2 laboratories. Recently, using an artificial (silicone rubber) membrane, the intra-laboratory and inter-laboratory variation of methyl paraben absorption was assessed in 18 laboratories (Chilcott *et al.*, in preparation). In their study, the CV values between laboratories were approximately 35 %, while the intra-laboratory variation averaged 10 %.

In the study presented here, the *in vitro* absorption of 3 compounds through human skin (9 laboratories) and rat skin (1 laboratory) was investigated. The compounds (testosterone, caffeine and benzoic acid) have a wide spread in their physico-chemical properties and have been recommended as reference compounds by the OECD (2000c). The studies were performed according to a very detailed protocol. Two participants were GLP-compliant while the other laboratories adhered to this quality system as much as possible. Analysis of samples from studies using non-radiolabeled test compounds was performed centrally in order to limit analytical variation and data analysis of all laboratories was carried out according to a study-specific Excel™ spreadsheet. Although the total recovery of the radioactivity at the end of the experiment was not always as high as required by the guidelines (100 ± 10 % for OECD and 100 ± 15 % for SCCNFP), all data were included in the calculations. Information on the mass balance is often lacking in the open literature, but we present these data in order to give a complete overview of the sources of variation when performing *in vitro* skin absorption studies.

Each laboratory performed three to five independent experiments with each compound. The reproducibility of the maximum absorption rate determinations within each laboratory was generally higher for benzoic acid than for caffeine and testosterone. The observation that the intra-laboratory variation in this study was generally higher than that observed for absorption of methyl paraben through a silicone rubber membrane (Chilcott *et al*., in preparation) may be explained by two important differences between the experimental designs. In the present study the compounds were applied at finite dose (25 □g/cm^2 of a solution of 4.0 mg/mL) while methyl paraben was applied at an infinite dose (1.0 mL/cm^2 of a saturated solution). In the case of a finite dose, it is critical to ensure that the entire skin surface is exposed to the small volume, which is technically demanding and may therefore explain some of the variation observed. Furthermore, the experiments presented here were performed with human skin, a biological rather than chemical membrane. In this respect it is important to note that

the variation in the barrier function between individuals and between anatomical sites has been reported to range between 2- and 6-fold, depending on the method of measurement (Schaefer and Redelmeier, 1996). Lee *et al.* (2002) reported an eight-fold variation on testosterone penetration through samples of human breast skin. The outliers observed by some laboratories may have therefore resulted from the choice of skin for that experiment. These results also highlight the need for consideration of inter-individual variability in skin penetration for effective risk assessment.

All laboratories assigned the absorption of benzoic acid through human skin the highest ranking of the three compounds (overall mean flux of 16.54 □g/cm^2/h). The absorption of caffeine and testosterone through human skin was similar, having overall mean maximum absorption rates of 2.24 □g/cm^2/h and 1.63 □g/cm^2/h, respectively. In 7 out of 9 laboratories, the maximum absorption rates of caffeine were ranked higher than testosterone. The absorption differences between the compounds fit well in with those reported in human volunteers, where the absorption of benzoic acid was found higher than that of caffeine and testosterone (Bronaugh and Franz, 1986). It should be noted, however, that the concentrations used in the *in vivo* study were not the same for each compound, which makes a direct comparison of the ranking to our results difficult.

One of the participating laboratories tested the absorption of compounds through rat skin instead of human skin. Rat skin is generally regarded as more permeable than human skin (ECETOC, 1993), although some cases to the contrary have also been reported (Hotchkiss *et al*., 1992). In the present study, no differences were observed between the mean absorption through human skin (all thicknesses included) and the one rat study for benzoic acid and testosterone. In contrast, for caffeine the maximum absorption rate and the total penetration through rat skin were clearly higher than the mean value for human skin. The observations on species-differences should be interpreted with some care, since the data on rat skin were limited to one laboratory.

One of the purposes of the study was to identify variables which have a large impact on the absorption data. When evaluating the data, it appeared that no consistent relation existed between the diffusion cell type (static or flow-through) and the absorption of the test compounds. These results are in line with previously reported data (Bronaugh and Stewart, 1984; Clowes *et al*., 1994; Roper *et al*., 1997). Moreover, no relation was observed between the absorption of the test compounds and the ratio between the volume of the receptor compartment and the exposed skin area of the diffusion cell, indicating that the cell design had not influenced the diffusion process.

A variable that is often considered critical is skin thickness. In the present study, skin thickness only slightly influenced the absorption of benzoic acid and caffeine. In contrast, the maximum absorption rate of testosterone was clearly higher in the laboratories using thin, dermatomed skin membranes. Testosterone is the most lipophilic compound, with a log Po/w of 3.32 (Table 1), and showed a higher presence in the skin membrane after 24 hour than the two other compounds (even though BSA was added to the receptor fluid in order to increase the solubility of testosterone). Parallel studies with caffeine and testosterone using full and dermatomed skin in the same flow-through system confirmed a greater influence of skin thickness on testosterone penetration (Wilkinson *et al*., in preparation). These studies therefore show that calculations of *in vitro* skin absorption of lipophilic compounds, based on receptor fluid levels only, should be treated with utmost caution and that information on the fate of the chemical in the skin is required to demonstrate whether it remained in the stratum corneum and/or in deeper layers of the skin.

In conclusion, our results indicate that the *in vitro* methodology for assessing skin absorption is relatively robust. A major effort was made to standardize the study performance, but, unlike in a formal validation study, not all variables were controlled. The variation observed may be largely attributed to human variability in dermal absorption and the skin source. For the most lipophilic compound, testosterone, skin thickness proved to be a critical variable.

Acknowledgements

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Table 1: Test substances

Test substance	**CAS no.**	**MW**	**log Po/w**
Benzoic acid (benzenecarboxylic acid)	65-85-0	122.1	1.83
Testosterone (4-androsten-17β-ol-3-one)	58-22-0	288.4	3.32
Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione)	58-08-2	194.2	0.01

Table 2: Details of source of human skin

Participant	**Number of donors**	**Post-mortem/ surgical waste**	**Sex and age donor**	**Body site**	**Skin thickness**
1. University of Newcastle UK	17	surgical waste	female (20 – 59 y)	breast	0.5 mm
2. Instituto di Medicina del Lavoro ITALY	6	post-mortem	male (67 – 90 y)	leg	0.7-0.9 mm
3. Universita di Trieste ITALY	7	Post-mortem	male, female (67-89 y)	abdomen	0.8-1.8 mm
4. TNO Nutrition and Food Research THE NETHERLANDS	6	surgical waste	female (28 – 69 y)	abdomen	0.7 mm
6. Imperial College London UK	3	surgical waste	female (29 – 50 y)	abdomen	0.9 mm
7. Health & Safety Laboratory UK	3	surgical waste	female (26 – 60 y)	abdomen	0.5-0.7 mm
8. University of Southern Denmark	22	surgical	female	breast,	0.7-1.1

DENMARK		waste	(16 – 68 y)	abdomen	mm
9. University of Erlangen-Nuremberg GERMANY	2	surgical waste	male, female (40 – 79 y)	breast, leg	0.9 mm
10. Huntingdon Life Sciences Ltd. UK	5	post-mortem	male, female (40 – 72 y)	abdomen, leg	0.3-0.4 mm

Participant no. 5 used rat skin

Table 3: Details of diffusion cell systems

Participant	**Diffusion cell type**	**Exposed skin area**	**Receptor compartment**	**Reference**
1. University of Newcastle UK	Flow-through	0.64 cm^2	Volume: 0.25 mL Stirrer bar: yes	Clowes *et al.* (1994)
2. Instituto di Medicina del Lavoro ITALY	Flow-through	0.95 cm^2	Volume: 3.5 mL Stirrer bar: yes	Reifenrath *et al*. (1994)
3. Universita di Trieste ITALY	Static	3.14 cm^2	Volume: 15 mL Stirrer bar: yes	Larese Filon *et al.* (1999)
4. TNO Nutrition and Food Research THE NETHERLANDS	Flow-through	0.64 cm^2	Volume: 0.2 mL Stirrer bar: no	Bronaugh and Stewart (1985)
5. Institut National de Recherche et de Sécurité, FRANCE	Static	1.76 cm^2	Volume: 5.15 mL Stirrer bar: yes	-
6. Imperial College London UK	Flow-through	0.32 cm^2	Volume: 0.4 mL Stirrer bar: no	Bronaugh and Stewart (1985)
7. Health & Safety Laboratory UK	Flow-through	0.5 cm^2	Volume: 0.35 mL Stirrer bar: yes	-
8. University of Southern Denmark DENMARK	Static	2.12 cm^2	Volume: 17.7 mL Stirrer bar: yes	Nielsen and Nielsen (2000)
9. University of Erlangen-Nuremberg GERMANY	Static	0.64 cm^2	Volume: 5.0 mL Stirrer bar: yes	Franz (1975)
10. Huntingdon Life Sciences Ltd. UK	Flow-through	0.64 cm^2	Volume: 0.25 mL Stirrer bar: yes	Clowes *et al.* (1994)

Figure 1: Overview of the maximum absorption rates of benzoic acid (blue), caffeine (red) and testosterone (white). ND is not determined.

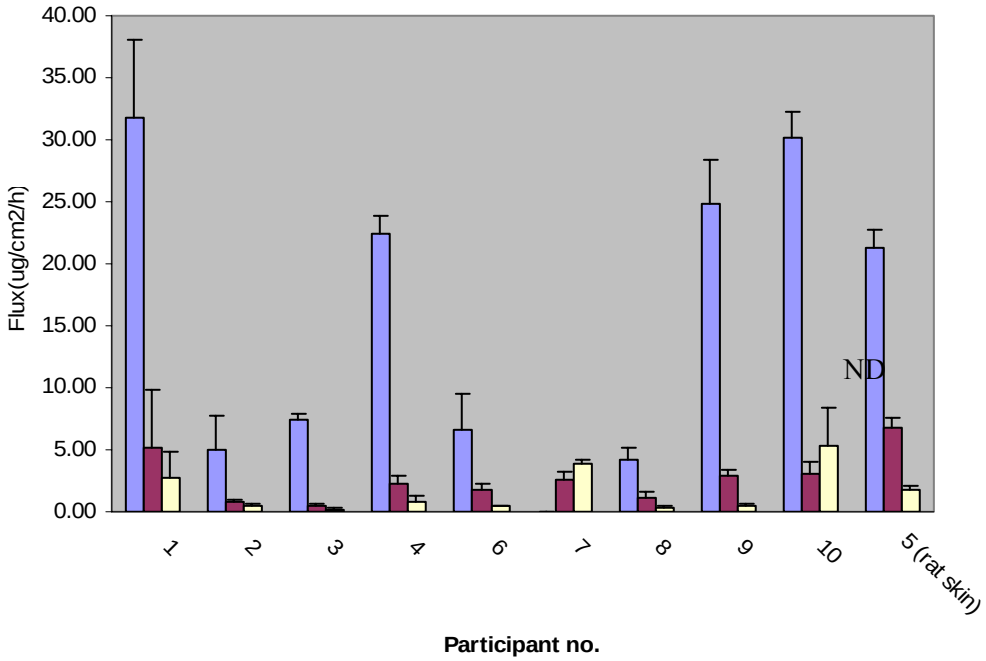


Figure 2: In vitro skin absorption of benzoic acid, expressed as percentage of the dose present in the receptor fluid (blue) or present in the receptor fluid + skin membrane (total penetration - red). ND is not determined.

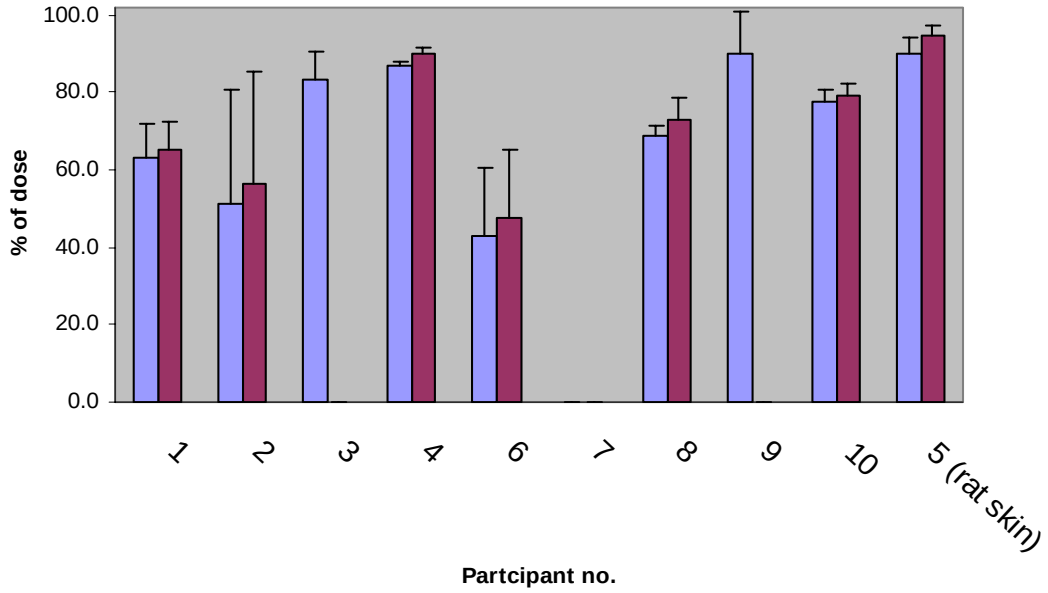


Figure 3: In vitro skin absorption of caffeine, expressed as percentage of the dose present in the receptor fluid (blue) or present in the receptor fluid + skin membrane (total penetration - red). ND is not determined.

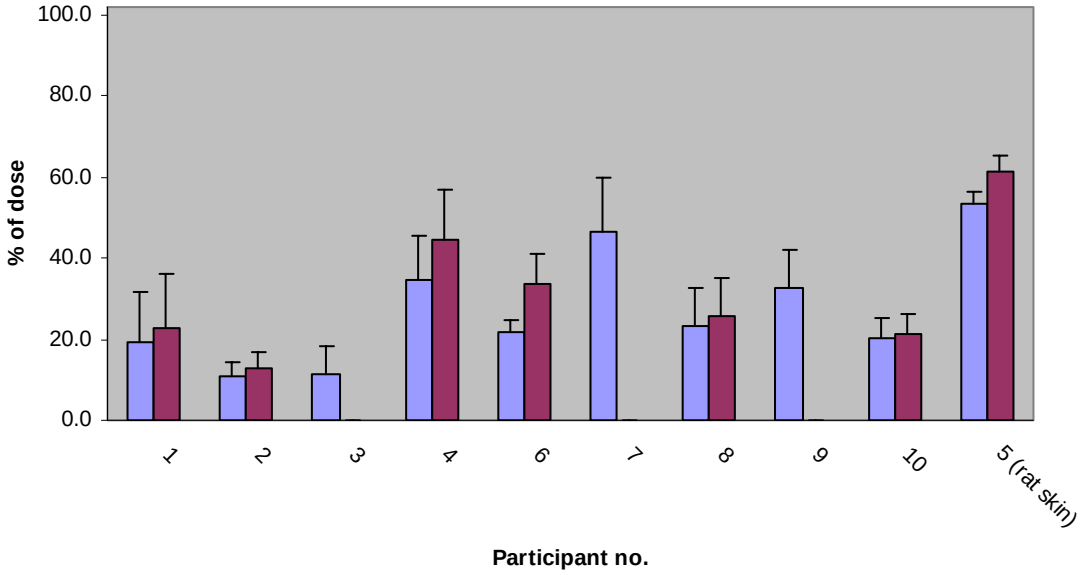
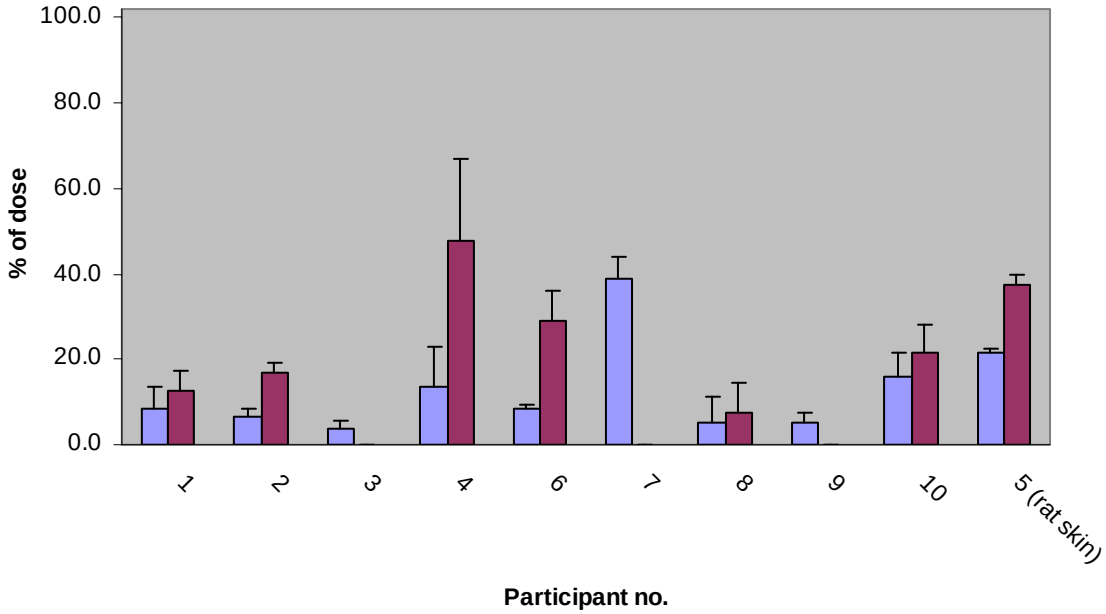


Figure 4: In vitro skin absorption of testosterone, expressed as percentage of the dose present in the receptor fluid (blue) or present in the receptor fluid + skin membrane (total penetration - red). ND is not determined.



Work Package 2 In vivo studies in volunteers

Coordinator and author Sanja Kezic AMC Holland

Objectives

The main objective of WP2 was to generate quantitative in vivo data mainly in humans that could be used for comparison with in vitro predictive methods and mathematical models. Furthermore, the in vivo studies aimed to provide new relevant data for risk assessment of dermal exposure to chemicals.

Summary

The in vivo studies were designed in the same manner (application mode, duration, concentration, vehicles) as the in vitro studies in WP1 and WP 3. The chemical of interest was applied to the skin of the forearm of the volunteer. The choice of chemicals studied was based on the physico-chemical properties of the chemical and its relevance for occupational exposure.

Dermal absorption in vivo was determined using two different approaches: biological availability methods and microdialysis. To enable comparison with literature data which were mainly obtained from studies with experimental animals, one participating institute conducted in vivo studies in the rat.

The percutaneous absorption of 50 % aqueous 2-butoxyethanol (BE) was determined by all participants. To investigate the effect of water on the percutaneous absorption of BE, three participants extended their study with additional BE concentrations. The other compounds studied were aqueous trichloroethylene (TRI) and xylene vapour (XYL) in human volunteers, caffeine, 0.05 % in human volunteers and rats and pyrene, benzo(a)pyrene and diethylhexylphthalate(DEHP) in rats.

In the studies based on biological availability, the compound itself or its metabolite was measured in exhaled air, blood or urine. To derive the permeation kinetics, a linear system dynamics method based on mathematical deconvolution was used. [4] In one study with 50% butoxyethanol data was fitted using Kinetica.

This method had been shown to be superior to the conventional methods since it provided additional information on the dermal kinetics enabling better comparison with in vitro data.

[4] Jakasa et al (2004) Percutaneous absorption of neat and aqueous solutions of 2-butoxyethanol in volunteers Int Arch Env Occup Health 77 79-84

An important aspect of the project was to assess variation in the determined values, including inter-laboratory variation, intra- and interindividual variation. The inter-laboratory variation estimated from the data on the permeability coefficient (K_p) of BE amounted to 45 % (CV) (mean 2.56; range 1.8-4.2). To estimate intra-individual variation, studies on BE and TRI were performed in each individual on two separate occasions. The results suggest that the intra-individual difference in percutaneous absorption in humans was small; the coefficient of variation (CV) for TRI and BE was 16 and 13 %, respectively. The interindividual variation was somewhat higher, the coefficient of variation amounted to 10, 35 and 22 % for TRI, XY and BE, respectively. [56]

One of the important advantages of the in vivo human experiments is that they provide data which has been directly derived in man. Human volunteer data is useful for risk assessment in parallel with in vitro human skin data (SANCO and SCCNFP documents). In the study on percutaneous absorption of BE it was shown that water increased percutaneous absorption of BE markedly. These findings are important for the health risk assessment of occupational exposure to BE, since BE is commonly used in mixtures containing water. Exposure to aqueous solutions of BE results in substantial skin absorption: assuming a 60 min skin contact of 1000 cm,^{2} dermal uptake would be four times higher than the pulmonary uptake of the 8-hour occupational exposure at the occupational limit value for BE of 100 mg m^{-3}. This clearly justifies the skin notation for BE and indicates the usefulness of human volunteer studies with biological monitoring to directly generate data for risk assessment. At present, biological monitoring programs are based on the determination of the concentration of free butoxyacetic acid in the urine however in our study we have shown that a substantial proportion of the butoxyacetic acid is as a conjugate. Considering only free butoxyacetic acid (BAA) will lead to erroneous estimation of the internal absorption. [7]

Microdialysis

The microdialysis technique was shown to be a suitable method for determining percutaneous absorption of BE, however this method was not appropriate for measuring the absorption of highly lipophilic molecule pyrene. The general limitation of this technique was the poor

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[5] Sanja Kezic[1] Astrid Janmaat[1]Jacob Krüse[2](),, Aart.C. Monster[1],Maarten.M. Verberk [1]PERCUTANEOUS ABSORPTION OF m-XYLENE VAPOUR IN VOLUNTEERS DURING PRE-STEADY AND STEADY STATE Toxicology Letters in press

[6] Percutaneous absorption of water solution of trichloroethylene in volunteers (DRAFT VERSION)

[7] S. Kezic, W.J. Meuling, I. Jakasa Free and total urinary 2-butoxyacetic acid following dermal and inhalation exposure to 2 butoxyethanl in human volunteers Int Arch Env Occup Hlth (2004) 77

recovery of the chemical through the dialysis tubing and obtaining an accurate estimate of the recovery of a chemical in the microdialysis fluid. In our study, the recovery was determined by comparison with metabolite excretion in the urine. Interestingly preliminary results indicated some oxidative metabolism of butoxyethanol locally in the skin during absorption.

Microdialysis gave similar estimates of flux for butoxyethanol to biological monitoring in the human volunteers The recovery in the volunteer studies was estimated using urinary butoxyacetic acid levels but this was not ideal in this study as limited urine samples were collected after the microdialysis study. [8]Microdialysis offers a useful adjunct to biological monitoring in which the amount diffusing through the skin can be locally determined rather then estimated from blood or urine profiles. Microdialysis also allows investigation of local metabolism during absorption. Similarly direct comparisons can be made in animals. In this project microdialysis was also compared with the in vitro approach using skin in a static cell in which microdialysis tubes had been inserted. Butoxyethanol, toluene and propoxur were studied. The presence of microdialysis tubing in the skin in vitro lowered the amount of material absorpbed into the receptor fluid but to different levels 25% , 40% and 50% of the total amount absorbed (receptor fluid plus dialysate) was present in the dialysate for toluene, butoxyethanol and propoxur respectively.[9] The disadvantages of the microdialysis approach was the poor recovery of the chemicals through the tubing. In the human studies the recovery appeared to be much higher in the in vitro experiments..

Percutaneous absorption of caffeine was determined in rats and in volunteers in vivo and in vitro in parallel. The results showed that rat skin overpredicted the percutaneous absorption of caffeine in the human skin, and that the extent of overprediction was dependent on the applied dose[10]. In contrast to pyrene, rat skin was less permeable for BE, however the promoting effect of water on the percutaneous absorption was similar to that observed in volunteers.

In vivo topical application studies in the rat were compared with in vitro rat skin measurements of absorption under the same conditions for butoxyethanol water mixtures, pyrene, benzopyrene and diethylhexylphthalate .(INRS)[11] Flux and Kp values were very similar for butoxyethanol mixtures whereas the flux through skin in vivo and Kp were underestimated by the in vitro studies. Similar differences were observed for caffeine particularly at the high dose (TNO)[12].

[8] Participant 10 Erlangen paper in preparation
[9] Participant 4 TNO and participant 10 Erlangen paper in preparation
[10] Participant 4 TNO paper in preparation
[11] participant 8 INRS paper in preparation
[12] participant 4 TNO paper in preparation

Conclusion

In vivo experiments were shown to be a valuable tool for validation of in vitro methods. In vivo data were of particular value for human risk assessment as they were directly usable. Furthermore important information on inter-individual variation in percutaneous absorption between individuals has been generated that can be related to the variation obtained in vitro. Using a method based on deconvolution, absorption parameters such as K_p, maximal flux and lag time could be derived enabling direct comparison with in vitro outcomes, and generating parameters suitable for use in predictive models.

Work Package 3 In vitro measurement of dermal absorption

Coordinator and author Faith M Williams Newcastle

Objectives

The results from the studies on robustness of the in vitro methods influenced the design of this work package which aimed to obtain robust in vitro data on chemicals in occupationally relevant situations and to generate new data to test existing predictive models. The aim was to generate data of occupational relevance using in vitro systems with human skin that have been evaluated for their robustness and ability to predict penetration in vivo when the same dose per unit area, volume per unit area and vehicle are applied. Some studies with rodents were conducted in parallel.

Methods

Guidelines on the design of in vitro studies based on the robustness study were circulated to participants (copy can be found at P51).[13] Studies were conducted using the in house diffusion cells (approx 50% division between static and flow through) with mainly 0.9% Na Cl. In some studies MEM or RPMI were used. The choice of receptor fluid was justified for lipophilic molecules and BSA was added to receptor fluid to ensure solubility of lipophilic compounds. Solubility was determined when not known. Finite doses of chemicals (generally 25ul/cm^2) as in robustness study were applied to the skin in an appropriate solvent for times that simulated occupational exposure. Some studies with commercial formulations were included. Infinite doses of near saturated aqueous solutions are also applied to generate data that could be used to develop QSARs based on the prediction of Kp values from molecular

[13] Guidelines for participants in WP3 (in vitro studies P51)

weights and the octanol-water partition coefficients. The list of chemicals studied is attached in table 1

These chemicals represented classes for which dermal absorption may pose a risk. They included glycol ethers, metals, pesticides, halogenated solvents, hydrocarbon solvents, polyaromatic hydrocarbons, pyridines, parabens, aromatic amines.

Table Chemicals studied

EDETOX

Chemicals Studied In Work Package 3

							Finite dose			Infinite dose		
Chemical	Participant	CAS No	LogP	Cell Type	Receptor Fluid	Vehicle	dose	time	volume	dose	time	volume
Paclobutrazol	Denmark	76738-62-0	3.2	static	0.9% NaCl+ BSA	2%EtOH, 0.9% NaCl	formulatiom			0.059 mg/ml	6-24	0.283 ml
Dimethoate	Denmark	60-51-5	0.7	static	0.9% NaCl+ BSA	2%EtOH, 0.9% NaCl	formulatiom			0.229	6-24	0.283 ml
Procloraz	Denmark	67747-09-5	4.4	static	0.9% NaCl+ BSA	2%EtOH, 0.9% NaCl	formulatiom			0.075	6-24	0.283 ml
Methiocarb	Denmark	2032-65-7	2.92	static	0.9% NaCl+ BSA	2%EtOH, 0.9% NaCl	formulatiom			0.045	6-24	0.283 ml
Pirimicarb	Denmark	23103-98-2	1.7	static	0.9% NaCl+ BSA	2%EtOH, 0.9% NaCl	formulatiom			0.048	6-24	0.283 ml
1 Ethoxxypropan-2-ol	Erlangen	1569-02-4	0.00	static	saline	50% in water				448 mg/ml	8	200 ul/cm2
Pyrene	Erlangen	129-00-0	4.88	static	saline	ethanol				4mg/ml	8	200 ul/cm2
DEGBE	Erlangen	112-34-5		static	saline					477 mg/ml		
Diethylhexylphthalate (DEHP)	France(rat)	117-81-7	7.6	static	0.9% NaCl+ BSA	Ethanol or neat	100-1200ug/cm2	24	25 ul/cm2			
N-methyl pyrrolidone	France	120-94-5	0.92	static	0.9% NaCl+ BSA	neat and aq solution	neat		25-400 ul/cm2	50% - 3% in aq		
Pyrene	France	129-00-0	4.88	static	RPMI+ BSA	Ethanol or acetone	4 mg/ml	24	50			
Benzo(a)pyrene	France (rat)	50-32-8	6.13	static	0.9% NaClor RPMI+ BSA	Ethano l or acetone	10	24	25			
Dibutylphthalate	France (rat)	84-74-2	4.5	static	0.9% NaCl+ BSA	neat	Neat	24	10			
2-Butoxyethanol	Newcastle, HSL, France (rat)	111-76-2	0.83	flow through	0.9% NaCl	Neat	Neat	8	25 ul/cm2	90.3 mg/cm2	4	200 ul/cm2
Caffeine	Newcastle, HSL, TNO	58-08-2	-0.07	Flow through	MEM	50% EtOH, water, butoxyethanol	100 ug/cm2	24	25 ul/cm2	19.4 mg/cm2	24	1000 ul/cm2
Testosterone	Newcastle	58-22-0	3.32	Flow through	MEM (Eagle) + 2% BSA	50% EtOH, water	0.53 – 100 ug/cm2	24	25 ul/cm2	21.2 ug/cm2		
Di methylene aniline (MDA)	Imperial	101-77-9	1.59	flow through	0.9% Na Cl	50/50 ethanol/water	312.5ug/cm2					
Trimethylamine	Imperial	75-50-3	0.16									
Diazinon	Newcastle	333-41-5	3.81	flow through	MEM (Eagle) + 2% BSA	water, acetone	200 ug/cm2	24 h	25 ul/cm2	20 ug/cm2	24 h	500 ul/cm2
Malathion	Newcastle	121-75-5	2.36	flow through	MEM (Eagle) + 2% BSA	Water, ethanol, xylene	71.5 ug/cm2	24 h	25 ul/cm2	71.5 ug/cm2	24 h	200 ul/cm2

Parathion	Newcastle, Siena	56-38-2	3.83	flow through	MEM (Eagle) + 2% BSA	water, ethanol, xylene	55 ug/cm2	24 h	25 ul/cm2	5.5 ug/cm2	24 h	200 ul/cm2
Triclosan	Newcastle	3380-34-5	4.76	Flow through	MEM (Eagle) + 2% BSA	water	0.25 ug/cm2	24 h	25 ul/cm2	10 ug/cm2	24 h	1000 ul/cm2
Benzo(a)pyrene	Siena, France (rat)	50-32-8	6.13	flow through	0.9% NaCl + 6% PEG 20							
Cobalt Chloride	Siena, Trieste	23670-59-9		flow through	0.9%NaCl 5% BSA pH 7.4			24hr	25ul/cm2			
Nickel Chloride	Siena, Trieste	37211-05-5		flow through	0.9%NaCl 5% BSA pH 7.4							
Sodium Chromate	Siena, Trieste	7775-11-3										
Propoxur	TNO	114-26-1	1.52	Flow through								
Diethylglycol buthylether acetate	Trieste	124-17-4	1.30	static		water	65g/l					
Methyl paraben	TNO	99-76-3	1.96	Flow thruogh	0.9%NaCl 5% BSA	water				645 mg/ml	24	
ethylparaben	TNO	120-47-8	2.47	Flow thruogh	0.9%NaCl 5% BSA	water				234	24	
Propyl paraben	TNO	94-13-3	3.04	Flow thruogh	0.9%NaCl 5% BSA					313	24	
Butyl paraben	TNO	94-26-8	3.57	Flow thruogh	0.9%NaCl 5% BSA					152	24	
Xylene	Trieste, TNO	1330-20-7	3.16									
Toluene	Trieste, TNO	108-88-3	2.73									
trichloroethylene	Trieste	79-01-6	2.42									
tetrachloroethylene	Trieste	127-18-4	3.40									
Dipropylenglycol methyl ether	Trieste	34590-94-8	-0.35	static		50% water	200ug/cm2					
Ethyleneglycol monoipropylether	Trieste	2807-30-9	0.08			50% water	200ug/cm2					
Ethyleneglycol monoisopropylether	Trieste	109-59-1	0.05			50% water	200ug/cm2					
Ethylenglycol monomethylether acetate	Trieste	110-49-6	0.1									
DEGBE	Erlangen	112-34-5	0.56	static	Saline	50% in water				500mg/ml	8h	200 ul/cm2
12 pesticides	HLS											

Several studies were stimulated by the results of the robustness studies and aimed to define the influence of factors such as skin thickness, the nature of the vehicle that can influence derived diffusion parameters for chemicals. Investigations of the effects of skin thickness on penetration to the receptor fluid and distribution were conducted for caffeine, testosterone and butoxyethanol [14](UNEW) and propoxur (TNO)[15] (previously described in this section). Many of the pesticides studied are lipophilic and attempts have been made to determine the influence of physicochemical factors (log P, molecular weight, solubility), occupational relevant design (dose, vehicle, finite versus infinite dose, mixtures and detergents) and the stratum corneum reservoir on dermal absorption. The penetration of caffeine through human skin in vivo and in vitro was compared with rat skin in vivo and in vitro (TNO) and metabolism of testosterone during dermal penetration through viable human skin in the flow through or static cells was determined (TNO) Penetration of benzopyrene, pyrene, DEHP and butoxyethanol was determined in the rat in vivo and using rat skin in vitro (INRS) Similarly studies in the rat in vivo were compared to rat skin in vitro by HLS for a series of pesticides applied in vehicle and formulation at low and high doses..

Results

Data has been generated for the dermal penetration of 60 chemicals through human skin in vitro. All the results are not presented n this section but the overall outcomes are summarised. Detailed reports from the individual participants are included in Section B.

Dermal penetration of pesticides through human skin in vitro under occupationally relevant conditions (finite doses) and from infinite aqueous doses was determined. The pesticides included organophosphates (parathion, diazinon and malathion) (UNEW, Siena), two carbamates (pirimicarb and methiocarb), prochloraz, paclobutrazol and dimethoate (Odense)[16] and a range of pesticide formulations (Huntingdon Life Sciences and TNO). Human versus rat in vitro data and rat in vitro versus in vivo studies were presented for the pesticide formulations and clearly showed an influence of skin reservoir. [17][18]The effects of vehicle and dose on the absorption of parathion and malathion was determined (UNEW) and the effects of a series of vehicles on the penetration of caffeine was shown to be large (HSL)

An accurate assessment of the potential for dermal penetration of metals has importance for risk assessment as absorption is generally accepted to be very low although many metals are sensitisers. Measurements of penetration of Chromium, Cobalt and Nickel through human

[14] Participant 1 UNEW paper in preparation
[15] Participant 4 TNO paper in preparation
[16] Nielsen JB, Nielsen, F, and Sorensen. JA (2004) In vitro percutaneous penetration of five pesticides – effects of molecular weight and solubility characteristics Ann Occup Hyg in press
[17] HLS Participant 12 paper in preparation
[18] TNO Participant 4 paper in preparation

skin with and without sweat were conducted at Trieste and Siena. [19],[20] Synthetic sweat promoted the absorption of metals through the skin.

Dermal exposure to glycol ethers and the influence of water mixtures promoting their absorption through skin has particular occupational relevance as these are widely used. Absorption through human skin of neat butoxyethanol (UNEW and HSL) and butoxyethanol 50% in water (eight groups), and rodent skin (INRS) was determined in vitro and shown to predict penetration in human volunteers (AMC) or rats in vivo (INRS). Absorption of a series of complex glycol ethers through human skin in vitro was also determined (Trieste[21] and Erlangen[22]).

INRS conducted parallel in vitro versus in vivo studies in the rat for diethylhexylphthalate, pyrene and benzopyrene penetration as described in previous section[23] .Parallel studies of exposure to xylene vapour and TCE in water with human skin in vitro and in human volunteers have been conducted (AMC and Trieste, HSL). Absorption and local metabolism and adduct formation of methylenediamine,dimethylamine and trimethylamine have been studied at Imperial College[2425] and the penetration of natural oils and their effect on skin integrity at Odense[26].

Some chemicals were selected specifically to generate data for QSARs to ensure data was available using our methodology and human skin for a range of log P values. Participants generated infinite dose data for aqueous solutions to parallel the occupationally relevant data. Infinite dose data for 21 chemicals ranging in log p between -0.35 and 4.42 was generated (See table). UNEW generated multiple time point mass in and mass out data with testosterone and infinite dose studies with testosterone and caffeine which could be used in mathematical modelling (See Section 4). When the data derived for the 21 new chemicals was added to the databases used by Flynn and Cronin the results fell within the current data set.

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19 Francesca Larese Filon, Giovanni Maina[§], Gianpiero Adami*, Marta Venier*, Nicoletta Coceani**, Rossana Bussani°, Marilena Massiccio[§], Pierluigi Barbieri* and Paolo Spinelli[§] IN VITRO PERCUTANEOUS ABSORPTION OF COBALT Int Arch Occup Environ Hlth (2004) 77 85-89

[20] Siena participant 2 paper in preparation
[21] Trieste participant 3 paper in preparation
[22] Erlangen participant 11 paper in preparation
[23] INRS Participant 6 paper in preparation
[24] Kenyon S, Bhattacharyya,J Benson CJ and Carmichael P (2004) Percutaneous penetration and genotoxicity of methylenediamine through rat and human skin in vitro Toxicology 196 65-75
[25] Kenyon, S, Carmichael, PL Khalaque S, Panchal S, Waring R, Harris, Smith RL and Mitchell SC (2004) The passage of triethylamine across rat and human skin Food Chem Toxicol 42 1619-2638
[26] Nielsen et al Effect of natural oils on penetration characterisitics paper in preapration

Table 3 contains log p data for 21 compounds measured using in vitro approach with human skin applying a saturated infinite aqueous dose

Compound	MW	LogP	Log(Kp)	CAS Number
DPGME	148.2	-0.3459	-4.05	34590948
EGNPE	104.15	0.08	-2.91	2807309
EGIPE	104.15	0.05	-3.26	109591
DEGBEA	204.27	1.3	-3.48	124174
EGMEA	118.13	0.1	-2.75	110496
Methiocarb	225.3	3.34	-2.69	2032-65-7
Pirimicarb	238.3	1.7	-2.56	23103-98-2
paclobutrazol	293.8	3.2	-2.50	76738-62-0
Prochloraz	376.67	4.4	-3.08	67747095
Trichloroethylene	131.39	2.42	-2.68	79-01-6
Tetrachloroethylene	165.83	3.4	-3.82	127-18-4
Caffeine	194.19	-0.07	-2.87	58-08-2
Parathion	291.26	3.83	-1.22	56-38-2
Malathion	330.35	2.36	-1.54	121-75-5
Butoxyethanol	118.18	0.83	-2.37	111-76-2
Testosterone	288.43	3.32	-2.02	58-22-0
Triclosan	290.5	4.76	-1.90	3380-34-5
Methyl paraben	152.2	1.96	-1.95	99-76-3
Ethyl paraben	166.2	2.47	-1.77	120-47-8
Propyl paraben	180.2	3.04	-1.81	94-13-3
Butyl paraben	194.2	3.57	-1.98	94-26-8

The usefulness of skin microdialysis for determining dermal penetration and comparisons with biological monitoring was conducted in human volunteers in WP2 (AMC, Erlangen.) in parallel, using the static cell, dermal microdialysis was compared with measures of penetration to the receptor fluid for toluene, propoxur and 50% butoxyethanol in water (Erlangen, TNO).Microdialysis tubing in the cell reduced the amount of material appearing in the receptor fluid by up to 50% for propoxur which was higher than might be expected form in vivo studies.

Discussion

Comparison of in vitro predictions of dermal absorption with measurements in volunteers and animals in vivo

Butoxyethanol was selected as a chemical for which parallel in vitro and in vivo studies were conducted both in human volunteers and rodents. The data following infinite dose application of 50% butoxyethanol/water was considered an example of an occupationally relevant situation. Two in vivo volunteer studies were conducted to cover inter laboratory variability (AMC and TNO) in which 50% butoxyethanol/water was applied to 40cm^2 of the forearm for 4 hours. As a reference exposure IV and inhalation exposures of a known input rate were performed by TNO and AMC respectively. The dermal absorption in both studies was standardised by comparison to an inhalation study (Jakasa et al 2004) as the dose in the intravenous study was too low to detect butoxyethanol or its metabolites. Predictions with dermatomed skin (flux 2.0 ug/cm^2/h) were close to the in vivo measurements of dermal flux (2.7 ug/cm^2/h) whereas with full thickness skin the flux to receptor fluid (0.8 ug/cm^2/h) underestimated the in vivo flux. For the rat studies using full thickness skin from young rat the flux to receptor fluid (1.8 ug/cm^2/h) was slightly higher than the in vivo flux (1.3 ug/cm^2/h)[27]. In conclusion with comparable exposure conditions the in vitro prediction was acceptable for in vivo flux of 50% butoxyethanol in water using the study design in EDETOX (See Table 4)[28][29]

The influence of water mixture on the flux of butoxyethanol was also well predicted by the in vitro approach with both human skin (figure 1)[30] and rat skin (Figure3). Comparison of the in vitro flux data indicated lower flux through human skin from neat butoxyethanol but higher flux at 75 and 50%. in water (figure 2).

Work Package 4 Modelling and Prediction of absorption

Coordinator and author S Corish Dublin TCD

SUMMARY of Work package 4

The EDETOX database was constructed at the University of Newcastle. It now contains critically assessed data on percutaneous penetration for more than 300 substances and is freely available at http://edetox.ncl.ac.uk, Details were presented in a poster[31] at the EDETOX Symposium at the Perspectives in Percutaneous Penetration (PPP) International Conference in France, April 2004.

[27] INRS Participant 6 paper in preparation
[28] Jakasa et al 2004
[29] Participant 4 TNO

[30] Wilkinson and Williams 2003
[31] 'A database of percutaneous absorption, distribution and physicochemical parameters', Sarah Soyei and Faith Williams.

Because of the complex nature of the skin membrane, the modelling of percutaneous penetration, the study of which was a major objective of EDETOX, has been largely confined to two approaches. The first of these is the development of Quantitative Structure Activity Relationships (QSARs) that relate the percutaneous penetration of a molecule to some of its physico-chemical properties. The second is the modelling of the macroscopic behaviour of the absorption process using diffusion equations or compartmental models that represent the vehicle, the sink the different layers of the skin to various levels of detail. This section of this report deals with an assessment of the currently available QSARs for skin penetration and the use of EDETOX data in this kind of modelling. A copy of a paper, recently published[32], that examines some of the most recently available and inclusive QSARs and makes some recommendations on the optimum form of QSAR and on the quality of data that is required to make reliable predictions of skin permeability based on such a QSAR is included. The use of a robust fitting technique to handle the data sets is examined as is the effects of incorporating the EDETOX data into the data sets used heretofore. The EDETOX Symposium at the PPP Conference included a poster[33] with the main results of this work.

A mechanistically-based mathematical model was developed during the project and that was used to interpret some of the data measured. This model enables the time courses of various variables to be simulated. These include the permeation rate and the cumulative adsorption levels present in the components of the system. In addition, modelling of a variety of exposure regimes, such as single and multiple dosing from different application forms can be handled. The model is also the subject of a poster at the PPP Conference[34]. The next section contains a report on the implementation of two other models. The first was a simple membrane model with a tractable analytic solution and the second implementation was of a pair of models developed by Anissimov and Roberts[35,36] that had to be developed in Laplace space and transformed numerically. These models were fitted to EDETOX data for Caffeine,

[32] 'Modelling Skin Permeability in Risk Assessment – the Future', D. Fitzpatrick, J. Corish and B. Hayes, *Chemosphere*, Vol 55. No. 10 (June 2004)
[33] 'QSARs for Percutaneous Penetration' D. Golden, D.Fitzpatrick and J. Corish
[34] 'Interpretation and Extrapolation of Deraml Permeation Data using a Mechanistically-based Mathematical Model', Jacob Kruse and Sanja Kezic.
[35] Diffusion modelling of percutaneous absorption kinetics: 1. Effects of flow rate, receptor sampling rate and viable epidermal resistance for a constant donor concentration, Yuri G. Anissimov and Michael S. Roberts, *J. Pharm. Sci*., 88, (1999), 1201-1209
[36] Diffusion modelling of percutaneous absorption kinetics: 2. Finite vehicle volume and solvent deposited solids**.** Yuri G. Anissimov and Michael S. Roberts, *J. Pharm. Sci*., 90, (2001), 504-520

2-butoxyethanol *(in vivo)*, Triclosan and testosterone: this work is also the subject of a PPP poster[37].

RESULTS and CONCLUSIONS

5.1.1 EDETOX Database

An extensive critically evaluated database on the percutaneous penetration of chemicals through the skin has been assembled and has been made publicly available at http://edetox.ncl.ac.uk. This database contains data from the literature as well as that generated in the project. The data fields were chosen to allow comparisons to be made regarding the experimental conditions under which the data were measured. It also includes information on the physico-chemical parameters that influence the adsorption process. Not all the data found in the literature satisfied the criteria of acceptability for the database.

5.1.2 Evaluation of Predictive Models

An extensive evaluation of existing predictive models for percutaneous penetration was carried out in the EDETOX project. These models currently take two principal forms: the first are QSARs that seek to establish a relationship between existing data and physico-chemical properties, typically a measure of the molecular size and the value of K_{ow} for the molecule, and predictions using mathematical models that are either diffusion type or based on a compartmentalised approach to the adsorption process. Both of these forms have been evaluated.

In the case of QSARs the largest published data sets were reanalysed and the effects of the selective removal of outliers assessed. It was concluded that these data had now been pushed to their limit and that there was no compelling evidence that the standard Potts and Guy formalism (based on molecular mass and K_{ow}) was significantly less good than any of the alternatives. WP4. was heavily dependent for its outcome on infinite and finite dose data generated in WP3.

The standardised data generated by the EDETOX project did not prove, when taken on their own, to be sufficient to establish a new statistically significant QSAR. The new data were therefore incorporated into the largest data set which was available to form a new data set containing a total of 181 compounds. Using the Potts and Guy QSAR form, these data, as

[37] 'Comparative Analysis of Non-steady State Data using Two Diffusion Models', J. Corish, D. Golden, S. Kezic and J. Kruse.

well as a number of sets reduced by the removal of selected outliers, were used to determine QSAR coefficients. The analysis techniques used in this process included both robust and standard least squares estimators. Although coefficients of determination (R^2) for all of the resulting QSARs were slightly lower than had been calculated for the corresponding data sets that did not include the EDETOX data. The similarity between the new standardised data set and existing data sets supported the use of existing QSARs.

Analysis of the results raised a number of further questions. The first relates to the quality of the data used to determine the QSAR coefficients. In particular, the values of K_{ow} and the appropriateness of the conditions under which they are measured, if experimental, or calculated. The second relates to the almost universal assumption of a linear relationship between the permeability and some set of physico-chemical parameters. The addition of the EDETOX data may suggest that a more complex relationship exist between these quantities. In this context we note the recent work on the use of artificial neural networks to determine a percutaneous penetration QSAR.

Use of QSAR in deriving dermal absorption data for risk assessment

Comparisons of Kp derived from QSAR based on infinite doses of saturated aqueous solutions with in vitro determination of absorption for finite aqueous doses and doses in occupationally relevant vehicles indicated the limitations of using the QSAR approach for predicting absorption for risk assessment. Studies in the project revealed the influence of vehicle and formulation on the observed flux. But have not defined the mechanisms. There is a need to further investigate this area and to derive QSAR models that take into account vehicle effects.

5.1.3 Development of a new mechanistic model

The EDETOX project has produced a novel mechanistic model that is particularly well suited to the modelling of dermal uptake following typical occupational exposure patterns. This makes possible modelling of repeated exposures and absorption from a variety of vehicles at both finite and infinite doses. It also includes *stratum corneum* and viable epidermis layers, with a parallel route to circumvent this barrier, and clearance of the penetrant by perfusion of blood. The physical characteristics of all of these are configurable. This model has been implemented in both ACSL and, more recently, in Berkeley Madonna. It has been used to fit EDETOX data, both finite and infinite, generated in Newcastle for testosterone and triclosan with sufficient early time points and gave similar predictions to Anissimov and Roberts. This model developed by J Kruse at AMC looks very promising and requires further validation with a more extensive data base of specifically generated absorption profiles.

5.1.4 Fitting of EDETOX Data by the Kruse Model

Data measured in the EDETOX project has been extensively fitted using the model described above. A new implementation, also done in the EDETOX project, of a finite dose model due to Anissimov and Roberts has been used to fit a subset of the same data. Where the results of these fitting processes have been compared they are within acceptable agreement. Both models were seen to successfully reproduce features of the absorption process such as the differences, expected on the basis of their lipophilicities, in the profiles of the mass in and mass out curves.

The fitting procedures have also shown that the relatively large number of parameters required describing the penetration process demand a commensurate large number of data points from which these parameters can be determined. When it is not possible to measure such a density of data points, dependable values of the parameters may be difficult to obtain.

5.1.5 Prediction of Percutaneous Penetration

These one-dimensional diffusion models can give a good qualitative understanding and description of the penetration process and, in suitable circumstances, can provide definitive values for the controlling parameters. They are promising but not yet a stage where they are generally applicable. Further work is needed to make them generally useful for the prediction of dermal penetration

5.2. Added value – future applications in the risk assessment of dermal exposures

The requirement to be able to assess the risks resulting from dermal exposures has increased recently and is likely to assume even greater importance and urgency in Europe with the introduction of the REACH regulations. These will impact, in particular, on the European chemical manufacturing industry and any assistance that can be provided in the determination of the risks associated with dermal exposure to chemical substances will be of great benefit to this sector of the European economy. It is clear that it will not be practicable to experimentally determine dermal penetration data for the very large number of chemical substances covered by the regulations and that, as a consequence, the development and use of predictive models that are acceptable to the regulators will be essential. In this regard the results presented in this report are particularly significant. First, the application of the models to the fitting of both finite and infinite dose measurements greatly improve our ability to interpret these kinds of experimental data and to extract accurate values for the parameters that govern the processes occurring during skin penetration. Two further significant advances follow from this ability to more accurately interpret experimental dermal penetration data. The first of these is that the more accurate values of transport constants, such as the permeability K_p, that result mean that the quality of the QSARs used to predict these data for unknown substances should also be more accurate and reliable. The second is that the

parameters determined in the fitting can then be used to calculate a wide variety of other quantities related to the detailed penetration processes in the same system. This has been illustrated where the quantities of the substance entering the skin have been calculated from parameters determined from measurements of the quantity leaving the skin to enter the receptor. The newly developed model particularly flexible and, once the parameters have been determined in the fitting process, it can be use to predict a wide variety of quantities relevant to occupational exposure patterns. As has been demonstrated these include intermittent and repeated exposures, with optional removal of the chemical remaining on the skin and a realistic modelling of the clearance, such as might be encountered during a normal working week or during leisure activities.

The development of these models and their testing through application to data measured in the EDETOX project has therefore resulted in considerable progress towards the development of a more reliable predictive tool for the estimation of the extent of dermal penetration of a chemical based on its physicochemical properties. What is now required is the optimisation and further development of these programmes into a more user-friendly package and its implementation, probably most usefully, on the web where it could be made available to those interested. This would require further support but the expertise required and, more crucially, the experience with the mathematical modelling and QSAR programmes, is available within the participants of the EDETOX project. We strongly recommend that this work should be undertaken without delay.

Work Package 5 Dissemination of results and discussion of Skin Notation

Coordinator Faith Williams author J B Nielsen Odense

The main focus of this work package was the preparation of the final report and organisation of the EDETOX day at the Predictions of Percutaneous Penmetration Meeting in April 2004.

Preliminary discussions were held on skin notation:

1) To describe the strengths and weaknesses of the present approach to the use of skin notations in different EU and non-EU countries.
2) To discuss possible ways to refine the skin notation in order to make it usable for prevention in occupational settings.

The skin notation was introduced almost 50 years ago as a qualitative indicator of hazards related to dermal absorption at work. A main complication is that the skin notation is now often used as an instrument for risk management. However, work-place exposures need to be assessed in quantitative terms, and a qualitative hazard indicator, such as the skin notation, is neither intended nor very useful for risk assessment or risk management. For this reason, some countries have developed

alternative criteria for assigning skin notations to industrial chemicals. Substantial discrepancies now exists between countries that otherwise have very comparable occupational exposure limits[38]. Moreover, up to one-third of all industrial chemicals with a TLV value now have a skin notation in many countries. The increasing number of chemicals with a skin notation and the large discrepancies between countries requires a new approach. This approach should incorporate variations in toxicity as well as penetration rates, which will allow a rating of the skin notation.
As risk assessment includes an assessment of exposure, which will vary over time as well as between scenarios, a skin notation will never be able to replace the risk assessment. Thus, a skin notation should relate to the potential for toxicity following relevant dermal exposure and be generic for the chemical in question. A refined skin notation may incorporate different degrees of toxicity and dermal penetration rates. We suggest a new approach to a semi-quantitative skin notation that will allow users to differentiate between chemicals with identical potentials for skin penetration but with different toxicities and between chemicals with equal toxicities but different penetration potentials. One of the new approaches is that the skin notation should relate to the product instead of the chemical. For example butoxyethanol will have very different penetration characteristics depending on whether it is in an aqueous solution or not. Therefore the skin notation would have to be different for the two products. Incorporating products instead of chemicals will also allow incorporation of the effect of penetration enhancers present in products.
The penetration characteristics as well as toxicity profile should be considered possibly using numeric scales to allow calculation of an integrated number. This area needs further consideration.

Conclusions and Recommendations from the EDETOX Project

Conclusions

Within this project the first large scale inter- laboratory comparison of *in vitro* dermal absorption methodology has been performed. It has shown that the *in vitro* method was reliable between laboratories and identified the factors contributing to intra and inter laboratory variability. Cell design was not identified as a major contributor to variability. It was concluded that variability between skin from different individuals was significant and contributed to the overall variability of the measurement of absorption. Therefore it was recommended that at least three samples of human skin be used and preferably six. The factors that contributed to the variability in the rate and extent of absorption between samples of skin from the same body site from healthy individuals were not fully defined and require further investigation. Skin thickness (whether full thickness or dermatomed)

[38] Nielsen JB and Grandjean P Criteria for skin notation in difference countries Am J Ind Med 2004 45 275-280

certainly contributed to variability but its importance varied between compounds, having the most effect on lipophilic molecules

In vivo studies in human volunteers are still the gold standard for obtaining dermal absorption data particularly if the dosage regime is designed to represent the actual in use exposure scenario. These studies have indicated how important parallel in vivo measurements are for validation of *in vitro* methods Inter laboratory volunteer studies have shown that inter individual variation in absorption between volunteers in vivo was up to three fold and this needed to be taken into account when relating in vivo to in vitro measures.

The choice of the biological exposure indicators for human volunteer studies was important and the use of sensitive analytical techniques with newly evaluated mathematical methods enable short exposure experiments at exposure levels far below the TLV which may be acceptable for compounds of known toxicity and extend the use of this approach.
In vitro predictions of flux and Kp through rat and human skin were reasonably similar to those determined in vivo there being an order of three fold differences. However the physicochemical properties of the chemical influenced how closely *in vivo* absorption could be predicted from *in vitro* studies. These studies have confirmed that if *in vivo* versus *in vitro* correlation data are available for one compound in a chemically related series, confidence in the *in vitro* data for other members is increased.

Limited studies were conducted in the rat to place the human data in context. *In vivo/ in vitro* correlations for the rat were better than for human for butoxyethanol but not for caffeine. These studies confirmed that for a particular chemical if in vivo/in vitro comparisons of penetration in the rat together with parallel in vitro measures using human skin were available it was possible to extrapolate to a figure for human dermal absorption in vivo.

Using the in vitro method tested for robustness, absorption data was generated for 60 chemicals either following a finite occupationally relevant dose or an infinite aqueous dose. Selection of dose and vehicle to represent the actual exposure were important when using *in vitro* approaches to generate relevant data for risk assessment: Full distribution data is important for interpretation of *in vitro* data for risk assessment. Distribution profiles determined in the rat were similar in vitro and in vivo. Stratum corneum reservoir *in vivo* was reflected by *in vitro* experiments

The results from this study do not indicate that data generated by the current model predictions can substitute for well designed absorption studies in vitro which represent the actual exposure scenario. This is mainly caused by the lack of understanding of the relationship between infinite dose and finite dose absorption profiles and by the fact that it is presently impossible to account for vehicle and formulation effects in the models. Flux values derived from the Kp predicted from QSAR generally overestimated absorption from a finite aqueous dose determined using an in vitro model, particularly for lipophilic molecules and high doses. The effect of vehicle or formulation could not be accounted for.

The studies described here confirmed that one-dimensional diffusion models can give a good qualitative understanding and description of the penetration process and, in suitable circumstances, can provide definitive values for the controlling parameters. However QSAR models are not yet at a stage at which they can be generally useful for the prediction of dermal penetration following a finite dose or take into account the effect of formulation. The recently developed QSAR models have not greatly improved on the Potts and Guy predictions. Infinite dose data generated for a further 21 compounds although insufficient to establish an independent QSAR data set was consistent with the existing data set supporting their use for model predictions.

Recommendations

During the three years that research has been carried out on this project in vitro skin absorption data has gained regulatory acceptance particularly for cosmetics and pesticides. The results from the project support the view that in vitro data provide a suitable model for the absorption of such chemicals in vivo

The approach of using in vitro methodology with human skin supports EU aims of developing alternative methods and reducing the use of animals.

The successes of the Dermal Network and subsequently of this project have demonstrated the need to maintain a network of laboratories in Europe with expertise and interests in aspects of dermal penetration.

The network can assist regulators setting standards and help the EU be abreast of innovation in dermal research; this will support its moves to adopt OECD Guidelines and

establish EU wide Guidelines. Outcomes from this project should influence refinement of Guidance documentation.

A database and web site have been established and these should be maintained and updated. These resources have already attracted interest and we recommend their use for research and as a source of evaluated data for the development and validation of predictive models, and for use in gathering data on related chemicals for the "weight of evidence" approach to risk assessment.

The EDETOX Workshop at the Predictions of Percutaneous Penetration Conference in April 2004, current publications and those in preparation have started the dissemination of the results. Circulation of this report and presentation of results at Meetings such as CEFIC Workshop, British Toxicology Society, and NIOSH etc will continue this process.

Findings on predictive models were presented at the QSAR 2004 meeting in Liverpool and will assist in strategies for the design of future predictive models. Outcomes from both predictive modeling and the design of in vitro protocols will contribute to the further development of protocols for generation of data suitable for improving existing QSAR models and building novel models for specific purposes, such as that proposed by CEFIC.

Refinement of the in vitro protocol and recommendations for further data generation to improve QSAR. There is a need for further investigations of the effect of vehicles formulation, multiple doses and mixtures on absorption. Data should be generated for QSARs that take into account the effect of vehicles.

Preliminary considerations of the current Skin Notation system have indicated the importance of an EU wide system which might be semi-quantitative. It is therefore recommended that further work be conducted in this area. (Nielsen et al 2004).

Further research is required to identify and define factors contributing to inter-individual variability on dermal absorption through the skin in order to be able to predict how permeable an individuals skin might be.

The importance of local metabolism and generation of toxic metabolites producing local skin damage such as irritation, sensitisation following penetration should be further investigated. This is an area that has received little attention in these studies although studies with aromatic amines (Imperial) testosterone (TNO) and butoxyethanol

(Erlangen) have shown detectable metabolism during absorption through viable skin in vitro and skin in vivo using microdialysis.

Future research should concentrate on development of finite dose models to mathematically define the profile of dermal penetration through skin both *in vitro* and *in vivo* at exposure relevant low level, short term doses. These should be extended to investigate the effects of multiple exposures, exposure to mixtures and vehicle and formulation effects on the dermal absorption profile of chemicals.
The model developed by J Kruse (Amsterdam) during this project can fit data for finite exposures if sufficient data points are generated over the early time phase of the absorption profile to define the lag phase and maximum flux. Although much of the available published absorption data contains insufficient time points for this approach but some of the data included in the EDETOX database http://edetox.ncl.ac.uk could be used. It is important to compare finite and infinite doses and effects of vehicles on the absorption profile in detail to provide a data base of information to validate this model. The use of the direct modelling approach should be pursued to assess its use for risk assessment in areas such as the new EU REACH regulations on use of chemicals.

This project has concentrated on the use of human skin in invitro systems as results with this can be directly extrapolated to risk assessment for humans. However with reduced availability of human skin for investigation due to ethical issues further investigations of alternatives such as pig or rodent skin should be initiated.

Exploitation and Dissemination of Results

1. A report including the methods and results generated during the EDETOX Project has been prepared for the European Union.
2. The EDETOX Workshop was held at the Predictions of Percutaneous Penetration (PPP) Conference in La Grand Motte in April 2004 (Programme attached).
3. Publications have been prepared for peer reviewed journals. A list of publications accepted for publication and those in preparation is included in the appendix to Section A together with copies of all accepted publications.
4. Presentations both oral and posters have been made at Scientific Meetings. (these are listed in the Appendix with copies of abstracts. Thirty abstracts were presented at the PPP Conference.

5. Members of the EDETOX project presented data at the recent CEFIC Workshop on methods to determine dermal permeation for human risk assessment in Utrecht Holland. Three members were on the steering group.
6. A copy of the final report will be provided to ECVAM
7. The database has been established at http://edetox.ncl.ac.uk for unrestricted access and it is hoped that new publications will be added to this in the future
8. A series of chemicals have been added to the database of chemicals used for QSAR.
9. A model that has the potential to predict flux following a finite dose has been designed but needs further evaluation

Policy Related Benefits

During the project we have established the robustness of in vitro methodology in a ring trial. Recommendations on methodology have been made which support OECD Guidelines and Guidance Documents.

Similarly the robustness and variability of in vivo volunteer studies was determined by parallel studies in three laboratories.

In support of the adoption of in vitro approaches to determining dermal flux comparisons of in vitro measurements and to data from human volunteer studies in vivo have been made. In particular extensive data has been obtained on the dermal penetration of butoxyethanol which should be available for risk assessment decisions and be considered in discussions of the Skin Notation for butoxyethanol.

The NIOSH in the US is presently revising their criteria for skin notation and it would be advisable for the EU to collaborate on this revision to achieve harmonised regulations

The outcome of this project is a recommendation to use human skin for dermal absorption studies thus reducing the use of animals in this area.

The output from this project should be considered together with that from other \EU funded projects such as RISKOFDERM to ensure that the appropriate data is available to support the development of parallel user friendly predictive approaches to generating data on exposure and the risk of absorption.

Achievement of Deliverables

All the deliverables for the EDETOX Project have been met.. Extensive data sets with several test compounds were generated, establishing the robustness of the in vitro technology. These

data have been published and can be used as reference for laboratories and authorities in the field of skin absorption and risk assessment. Special attention has been dedicated to the effect of skin thickness, which was found to be one of the most critical variables. Based on the outcome of the WP1 studies, guidance was given on partial standardizing of studies in WP3.
Similarly all the Deliverables of WP2 were achieved. In vivo data on dermal absorption of 2-butoxyethanol (25, 50, 90 and 100 %), trichloroethylene, xylene, caffeine have been generated and in vivo data on dermal absorption of 50 and 90 100 % 2-butoxyethanol have been generated using microdialysis method. Parallel in vitro-in vivo data have been generated in rats for 25, 50, 90 and 100 % 2-butoxyethanol, pyrene, benzo(a)pyrene and diethylhexylphthalate. All data obtained from in vivo experiments were used for the comparison with in vitro data The results of the trichloroethylene and xylene studies which were performed for different exposure durations were used for development and optimisation of diffusion models (WP 4). WP3 achieved its deliverables by generating extensive absorption data on the dermal penetration of chemicals from doses which were directly relevant exposures using the methods standardised in WP1. In vitro approaches were used to investigate the effects of vehicle and dose on dermal absorption and the influence of physicochemical properties on dermal penetration. In parallel was generated using aqueous inflate doses whixh extended the existing data base for QSAR modelling . In WP4 an extensive critically evaluated database on the percutaneous penetration of chemicals through the skin has been assembled. An extensive evaluation of existing predictive models for percutaneous penetration was carried out. In the case of QSARs the largest published data sets were reanalysed and the effects of the selective removal of outliers assessed. EDETOX data were incorporated into the largest data set which and this simultaneously updated wherever improved data were available to form a new data containing a total of 181 compounds. The EDETOX project has produced a novel mechanistic model that is particularly well suited to the modelling of dermal uptake following typical occupational exposure patterns. . It has been used to fit EDETOX data, both finite and infinite, as well as data from other sources. These one-dimensional diffusion models can give a good qualitative understanding and description of the penetration process.The deficiencies of the current Skin Notation has been discussed. A final report has been prepared and a Workshop organised to disseminate results and a series of papers are in preparation. Several areas have been highlighted in which research should be extended.

Guidelines for conduct of in vitro studies in Work Package 3 to generate data on a range of chemicals

Critical points from preliminary consideration of robustness study

1. Inter skin variability was significant. There is a need to use as many samples of human skin as possible. The factors contributing to this variability are not fully defined. The age of the donor and whether skin was fresh or frozen, from operation or from cadaver may contribute.

2. It is important to have evidence of barrier integrity following preparation of skin membranes.

3. Cell design was less of an influence although flow rate in flow through systems can influence absorption rates.

4. Skin thickness certainly contributed to variability. This need to be tightly defined

Study proposal Outline

Each participant should prepare an outline of the studies they intend to conduct for WP3. A preliminary submission has been sent to the Co-ordinator and was presented at the Amsterdam meeting. To ensure that studies are comparative and will achieve the aims of the project further details should be prepared at this stage.

Questions that should be addressed:

1. Is the study novel? Have you or someone else done it before?

2. Will it be publishable in its own right?

3. Is your proposed study within the guidelines and deliverables of WP3?

4. Will your study help WP4?

5. Is your study design occupationally relevant?

6. Have you included appropriate quality controls?

WP3 Guidelines

Diffusion cells

Each participant will use the diffusion cell established in their laboratory and used in the WP 1 standardisation exercise.
(See WP1 Protocol)

Skin membranes
As for WP1

Participants can make appropriate decisions regarding skin thickness.
Choices: whole skin (less than 1mm but actual thickness must be known), skin 500u, skin dermatomed (250-350u), epidermal membranes

Human skin

Record thickness of each piece of skin used
Number, age, sex of donors for each experiment and for which cells
Body area source of skin
Date of collection, length of storage, method of storage, temperature of storage

Specific attention must be applied to storage and freshness of skin for metabolism studies. Those doing metabolism studies are discussing an appropriate metabolic marker

Rat skin

Fresh skin should be used

Receptor fluid

This should be selected by each participating laboratory. It can either be saline (+/-) BSA as for WP1 or the receptor fluid used in house. The choice of receptor fluid should be justified.
It is essential to obtain evidence of solubility of the chemical of interest in the receptor fluid directly or using EPA programme to predict.

Experimental design

- The test chemicals can be applied as an infinite dose or finite dose depending on the study. Choice of vehicle may be agreed with WP3 sub groupings and might be water, basic solvent eg acetone or ethanol or occupationally relevant formulation

- ***Variables*** : time on skin, vehicle, dose, volume, multiple doses

- Occlusion or non occlusion will be selected as appropriate for the study

- Because of the variability between pieces of human skin up to 6 human skin membranes per chemical/ study would be optimal with results collected in one or two parallel studies. However this may not be practical and at least three skins should be used.

Standardisation

Absorption
Determine the absorption of an infinite volume of aqueous saturated solution of the chemical of interest plus one of the WP1 standards. - Measure the concentration of the saturated solution at beginning and end of experiment. Make observations about evaporation change in concentration with time.

Metabolism
Explore use of 14C testosterone metabolism to prove that skin is viable and has metabolism capacity. Only possible for some laboratories.

Distribution

Surface swab either by in house method or as WP1 guideline- with justification
Full distribution studies to be conducted to give surface wash, cell contamination, evaporated material, stratum corneum, epidermis? Dermis, receptor fluid levels and thus total recovery. This is not so easy with cold assays and participants should aim to get as much information as possible.

Collection of receptor fluid samples- as many time points as possible to fully define the absorption profile. Adapt sampling times to suit study design and nature of chemical under investigation.

Analysis of results
Results should be analysed using the spread sheet prepared by TNO for WP1
Output should include Lag time, steady state flux (time over which determined), Absorption to receptor fluid (absolute and as percentage of applied dose), absorption beyond stratum corneum (receptor fluid plus that in skin but not stratum corneum) with time. Distribution data.

The aim is that data acquired during WP3 will be added to the database for access by all participants. Full details of all studies will be required. It is often not easy to extract appropriate information from published literature as many important details are omitted.

A checklist of information for in vitro studies is attached.

General

Experiment number	
Date	
Persons involved	
Goal of experiment	

Experimental conditions

Occlusion (yes/no)	
Name test compound	
Vehicle	
Concentration (mg/mL)	
Volume applied (mL/cm^2)	
Name reference compound	
Vehicle	

Concentration (mg/mL)	
Volume applied (mL/cm^2)	
Mass applied	
If radio labelled countsapplied(	
Specific activity	

Skin membrane

Species	
Number of donors (at least 3)	
Donor information (sex, age, body	
Thickness (maximum 1 mm)	
Fresh/frozen+ temp (storage time)	
Date/time of collection	
Method of preparation	

Diffusion cells and receptor fluid

Diffusion cell type	
Skin area	
Receptor fluid composition	
Volume of receptor compartment	
Solubility of compound in receptor fluid	
Receptor fluid sample size (static cells)	
Flow rate (flow-through cells)	

Membrane integrity test

Test type	

Analysis

Sample preparation	
Analytical method	
Comments on vehicle evaporation, concentration of application	
Comments on results- anything unexpected	

Faith Williams, Han van der Sandt

List of Publications- full papers

Williams Faith M. Evaluation and predictions of dermal absorption of toxic chemicals *International Archives of Occupational and Environmental Health* 2004, 77, 150-151

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Francesca Larese Filon, Giovanni Maina[§], Gianpiero Adami*, Marta Venier*, Nicoletta Coceani**, Rossana Bussani°, Marilena Massiccio[§], Pierluigi Barbieri* and Paolo SpinelliIn vitro percutaneous absorption of cobalt *International Archives of Occupational and Environmental Health*(2004) 77 85-89

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Kezic et al Percutaneous absorption of water solution of trichloroethylene in volunteers. Paper in preparation

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Percutaneous Penetration studies for risk assessment Faith M Williams, , International Conference of Occupational and Environmental exposures of skin to Chemicals: Science and Policy 2002

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G.Adami, F.Larese, M.Venier, G.Maina, E.Reisenhofer, P. Barbieri: "Assorbimento percutaneo di polveri di cobalto: risultati di uno studio realizzato nell'ambito del progetto europeo EDETOX", XXI Congresso Nazionale della Società Chimica Italiana, Torino 22-27 giugno 2003, Atti, Vol.1, AN-CO-057 (2003).

F. Franco, "Valutazione dell'assorbimento percutaneo di glicoleteri", degree thesis, 2003.

Papers in preparation

Corish et al An extended QSAR database. in preparation

Nielsen JB et al Effect of natural oils on penetration characteristics Paper in preparation

Kruse et al A new mechanistic model for the prediction of percutaneous absorption of finite and infinite doses in preparation

Wilkinson et al Effect of vehicle and concentration on the percutaneous penetration of pesticides

Williams et al Butoxyethanol: its dermal penetration profile from neat and aqueous solutions using in vitro and in vivo data

Williams et al How well can one extrapolate predictions with skin in vitro to in vivo absorption? Are the data reliable?

Wilkinson et al Transdermal electrical resistance and absorption measurements in a flow through cell in preparation

G. Korinth, T. Wellner, I. Jakasa, S. Kezic, J. Krüse, K.H. Schaller Assessment of percutaneous absorption of 2-butoxyethanol by microdialysis in volunteers. Paper in preparation

WJM Maas, JJM van der Sandt,and J Korinth Percutaneous absorption of toluene, 2-butoxyethanol and propoxur determined using the microdialysis technique in vitro). Paper in preparation

Programme for EDETOX Symposium
Predictions of Percutaneous Penetration La Grande Motte 2004-
At www. ppp conference .org

THURSDAY 15th April

09:00 Introduction to EDETOX Symposium
Faith M Williams (Coordinator EDETOX)

09:10 In vitro measurements of dermal penetration in man
Han van de Sandt , TNO, The Netherlands

09:45 Human in vivo studies of dermal penetration; their relation to in vitro predictions
Sanja Kezic, Amsterdam, The Netherlands

10:20 COFFEE

10:50 Modelling percutaneous penetration
John Corish, Dublin, Ireland

11:25 Percutaneous penetration of occupationally relevant chemicals
Faith M Williams, Newcastle, UK

12:00 LUNCH

14:00 Conclusions and recommendations
Faith M Williams, Newcastle, UK

14:15 Use of percutaneous absorption data in risk assessment
Jon Heylings, Alderley Park, UK

Short presentations (5 x 15 minutes)

14.45 Percutaneous absorption of malathion and parathion in ethanol and xylene vehicles.
SC Wilkinson, Newcastle, UK

15.00 In vitro percutaneous absorption of metal powders.
M Venier, Trieste, Italy

15.15 Assessment of percutaneous absorption of 2-butoxyethanol by microdialysis in volunteers.
G Korinth, Erlangen, Germany

15.30 Efficiency of microdialysis for the assessment of percutaneous absorption varies between test compounds.
WJM Maas, Zeist, The Netherlands

15.45 A semi-quantitative approach to the skin notation.
JB Nielsen, Odense, Denmark

16:00 COFFEE

16:30 *POSTER VIEWING*

Influence of vehicle on chemical penetration through human skin in vitro
IP Dick, K Jones and J Cocker

In vitro skin absorption – can it be used in isolation for risk assessment purposes?
J O'Connor and S Cage

Determination using HPLC of partition and distribution coefficients, for use in transdermal modelling
D O'Neill and D Fitzpatrick

Qsars for percutaneous penetration
D Golden, D. Fitpatrick and J Corish

Comparative analysis of non-steady state data using two diffusion models
J. Corish, D. Golden, S. Kezic and J. Krüse

Interpretation and extrapolation of dermal permeation data using a mechanistically based mathematical model
J. Krüse and S. Kezic

Determination of the percutaenous absorption of polyethylene glycols of different molecular weights in volunteers by tape stripping
I. Jakasa, S. Kezic and F. Calkoen

Efficiency of microdialysis for the assessment of percutaneous absorption varies between test compounds
WJM Maas, JJM Van De Sandt and G. Korinth

Percutaenous absorption of pyrene using in vitro microdialysis
T Wellner and G Korinth

Assessment of percutaneous absorption of 2-butoxyethanol by microdialysis in volunteers
G. Korinth, T. Wellner, I Jakasa, S Kezic, J. Krüse and KH Schaller

In vitro percutaneous absorption of metal powders
M Venier, F Larese, G Adami and G Maina

In vitro percutaneous absorption of metals
P Sartorelli, L Montomoli, F Cioni and AG Sisinni

Percutaneous penetration of five pesticides – effects of molecular weight and solubility characteristics
JB Nielsen, JA Sørensen and F Nielsen

A semi-quantitative approach to skin notation
JB Nielsen, P Sartorelli and P Grandjean

Transdermal absorption of fentanyl from drug delivery plasters
RH Larsen, F Nielsen, JA Sørensen and JB Nielsen

Cosmetic oils affect the integrity of human skin
JB Nielsen

Percutaneous absorption of malathion and parathion in ethanol and xylene vehicles
SC Wilkinson and FM Williams

Influence of skin thickness on percutaneous penetration in vitro
SC Wilkinson, WJM Maas, JB Nielsen, LC Greaves, JJM Van De Sant and FM Williams

A database of percutaneous absorption, distribution and physicochemical parameters
S Soyei and F M Williams

18:00 EDETOX Reception

FRIDAY 16th April

"TOXICITY AND REGULATORY"
Contributed Papers Session
(Chair: Jon Heylings, Alderley, UK)

09.00 Development of a repeat dose in vitro skin penetration model
Ruth Pendlington, Colworth, UK

09.15 Determination using HPLC of partition and distribution of coefficients for use in transdermal modelling
Dara Fitzpatrick, Cork, Ireland

09.30 In vitro percutaneous absorption of metals
Petro Sartorelli, Siena, Italy

09.45 Cosmetic oils affect the integrity of human skin
J.B.Nielsen, Odense, Denmark

10.00 COFFEE

10.30 *TOPICAL DISCUSSION:*

"Design of Relevant Skin Permeation Guidellines"
Discussion Leaders:
Wim Meuling, Kenneth Walters and David Esdaile