EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Institute for Health and Consumer Protection

Global Net on "CONSUMER EXPOSURE MODELLING"

Report of the Workshop no. 1

on

Dermal Transfer and Penetration Algorithms

20-21 June 2005, Intra (Italy)

Editors: *Stylianos Kephalopoulos, Joop J. van Hemmen, Katinka van der Jagt*

2006 EUR 22521 EN/1

The mission of the Institute for Health and Consumer Protection is to provide scientific support to the development and implementation of EU policies related to health and consumer protection. The IHCP carries out research to improve the understanding of potential health risks posed by chemicals, biocides, genetically modified organisms, contaminants released from food contact materials and consumer products.

European Commission
Directorate-General Joint Research Centre
Institute for Health and Consumer Protection

Contact information
Address: Via E. Fermi 1, TP 281
E-mail: stylianos.kephalopoulos@jrc.it
Tel.: +39 0332 78 9871
Fax: +39 0332 78 5867

http://www.jrc.cec.eu.int/pce/

EUR 22521 EN/1
ISBN 92-79-03672-6B B
ISSN 1018-5593

Luxembourg: Office for Official Publications of the European Communities

Printed in Italy

Global Net on "CONSUMER EXPOSURE MODELLING"

Proceedings of the Workshop no. 1 on

Dermal Transfer and Penetration Algorithms

20-21 June 2005, Intra (I)

Workshop Coordinator: ***Stylianos Kephalopoulos***
Workshop Moderator: ***Joop J. van Hemmen***
Workshop Rapporteur: ***Katinka van der Jagt***

TABLE OF CONTENTS

Global CEM Net Report of the Workshop no. 1 on
"*Dermal Transfer and Penetration Algorithms*", Intra (Italy), 20-21 June 2005

PREFACE

The Exposure Modelling Sector of the Physical and Chemical Exposure Unit, Institute for Health and Consumer Protection of the European Commission's Joint Research Centre, organized a series of five specialized Workshops on ***"Consumer Exposure Models Inter-comparison (Phase II) – Framework/Policy and Research/Science major issues"***. These workshops, were held in June 20-24, 2005, in Intra (Italy), and constituted the top event of the activities of the Global Net on "Consumer Exposure Modelling" for 2005, a growing consortium of expert model developers and users from Europe, America, Canada and Asia, aiming at harmonizing and validating existing consumer exposure models on the basis of common procedures and protocols. This activity is contributing to the consumer exposure assessment efforts of the PCE Unit, supporting the EU General Product Safety Directive (*2001/95/EC*) and providing technical support to aspects of REACH (*Registration, Evaluation and Authorisation of CHemicals*).

During the first Global CEM Net Workshop on "*Consumer Exposure Models Inter-comparison (Phase I) – The state of the science and research needs*" held in Ispra, on 26-27 of October 2004, the need on focusing on five major topics was identified concerning model harmonization and validation. A series of five Workshops has been then organized in June 2005, based on the draft agendas prepared in the first Global CEM Net Workshop, dealing with the following five major topics:

<u>Research/Science</u>
Workshop no. 1 – "**Dermal transfer and penetration algorithms"**
Monday 20th to Tuesday 21st of June, 2005
Moderator: J. J. van Hemmen
Rapporteur: K.E. van der Jagt
Workshop no. 2 – "**Source characterization, transport and fate"**
Monday 20th to Tuesday 21st of June, 2005
Moderator: M. Jayjock
Rapporteur: A. Arvanitis

<u>Framework/Policy</u>
Workshop no. 3 – "**Exposure modelling framework/model management issues"**
Wednesday 22nd of June, 2005
Moderator: M. Jantunen
Rapporteur: A. Arvanitis

<u>"In-between"</u>
Workshop no. 4 – "**Exposure-related data"**
Thursday 23rd of June, 2005
Moderator: J. van Engelen, C. Money and P. Price
Rapporteur: A. Arvanitis
Workshop no. 5 – Scenario development
Friday 24th of June, 2005
Moderator: J. van Engelen
Rapporteur: A. Arvanitis

The **Workshop no. 1 on** "**Dermal Transfer and Penetration Algorithms"** held on Monday 20th and Tuesday 21st of June 2005.

The general rationale of this workshop was:

> *Many chemicals pose potential problems upon human dermal exposure. This requiresan estimate of dermal uptake, based on experimental data or mathematical modeling for risk assessment purposes.*

This specific workshop (Dermal transfer and penetration algorithms) addressed two general areas separately; viz., **transfer to the skin** and **penetration algorithms.**

The purpose of this workshop was:

> *1. To survey and discuss the general state-of-the-science of the methodology for assessing dermal penetration.*
> *2. To identify recommendations for next steps in modelling dermal exposure to consumer products and then prioritise the recommendations for future research.*

The focus of the workshop was not on specific substances but on the identification and development of general modelling constructs capable of describing the relevant factors for the multitude of substances impacting and penetrating human skin.

The expected duties of and opportunities for the participants have been to:

1. *Provide feedback and material to the Workshop report to be drafted by the Moderator before, discussed during and finalised after the Workshop.*
2. *Formally or informally present relevant research that they have done or have specific knowledge of, relative to these two general areas of study.*

This was done by covering at least the following issues:

a. How to use JRC EIS-Chemrisks "ExpoData" to help drive research needs?
b. How to address regulatory policy, specifically EC Guidance Document on Dermal Absorption dated 19 March 2004?
c. Considering the tiered approach to modelling dermal exposure, what sort of approach is necessary? A simple approach to screen many chemicals or a refined approach to estimate chemical specific dermal exposure?
d. What are the data needs for modelling dermal exposure to consumer products?
e. Which dermal exposure models are readily available and documented (either separate or integrated)?
f. How to address dermal uptake (experimental and model) uncertainty and variability?
g. How to "compare or corroborate" model predictions to experimental results?
h. What are the top priority dermal exposure chemicals/products/scenarios?

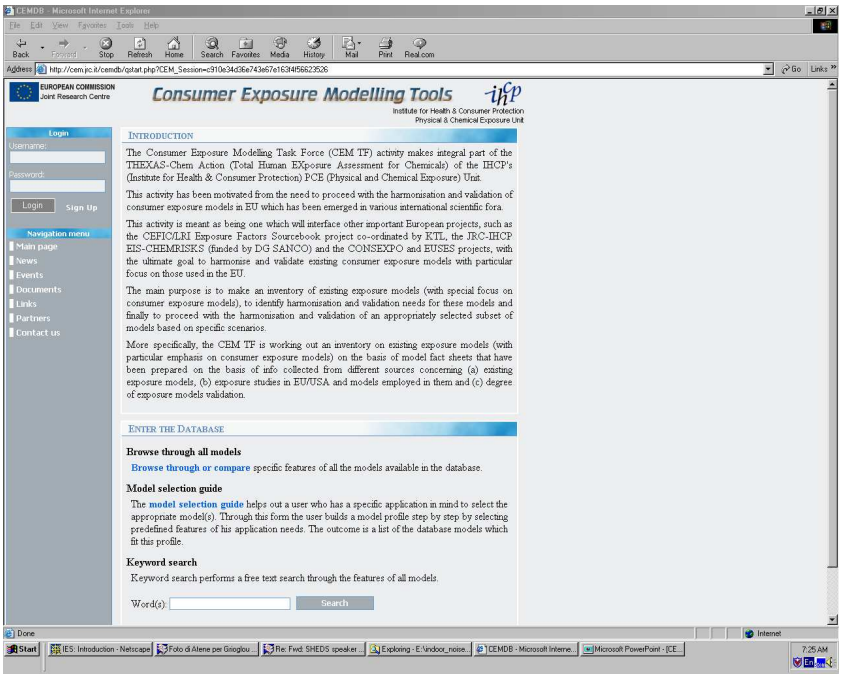
In previous workshops, formal presentations in the plenary session by the participants have significantly helped to set the tone for subsequent discussions. As such, participants were encouraged to present their

(and others) work. They were also kindly asked to advise the Workshop Moderator concerning the topic of the presentations and time required. The JRC coordinator and the Workshop Moderator have in turn planned the workshop potentially balancing the advantages of these presentations with the time available.

Since this specific workshop followed straight after the workshop on a similar subject took place at the OEESC-2005 (in Stockholm, mid June 2005), several of the participants of this Workshop may be at both workshops. This means that the results of the Stockholm workshop were presented in this Workshop and taken further ahead.

The report of this Workshop as well as other related documentation could be downloaded from the following Global CEM Net Website:

http://cem.jrc.it/cemdb/qstart.php



Dr. Stelios Kephalopoulos (***Global CEM Net Coordinator***)
Dr. Joop J. van Hemmen (***Global CEM Net Workshop no. 1 Moderator***)
Dr. Katinka van der Jagt (***Global CEM Net Workshop no. 1 Rapporteur***)

Global CEM Net Report of the Workshop no. 1 on
"*Dermal Transfer and Penetration Algorithms*", Intra (Italy), 20-21 June 2005

INTRODUCTION

The Global Net on "Consumer Exposure Modelling" is sponsored by the Physical-Chemical Exposure Unit of the Joint Research Center of the European Union in Ispra. It focuses on development, and international harmonization and validation of consumer exposure modeling approaches.

In the context of a series of five workshops organised by the Global CEM Net in June 2005, a workshop was held that focused on the skin absorption of chemicals "Dermal transfer and penetration algorithms". In fact this workshop can also be seen as a follow-up and further extension of a workshop held at the international Occupational and Environmental Exposures of Skin to Chemicals Conference in June 2005 at the Karolinska Institute campus in Stockholm, Sweden. For that workshop a preliminary white paper was prepared, as well as a series of statements (see *Appendix 1*) that formed the basis of the discussions.

Prof. Richard Guy (Method development and modelling to characterize penetration, absorption, dose, and local effects resulting from dermal absorption) and Dr. Nick Warren (Bayesian and probabilistic exposure modelling) gave plenary key note lectures for the conference, whereas Prof. Annette L. Bunge (Quantitative risk assessment), Dr. John Cherrie (Dermal exposure and uptake of chemicals for systemic risk assessment) and Dr. Derk Brouwer (Spatial and temporal variability of dermal exposure) introduced some relevant issues to stimulate the two-hour workshop discussions in order to bring together the scientists working on dermal exposure and those working on dermal penetration.

For the workshop held two weeks later in Italy, the preliminary white paper (*Appendix 2*) was essentially the same as for Stockholm and formed more or less the basis as well as the boundaries for discussions. The statements prepared for the Stockholm workshop were also used for the present workshop. The workshop was on invitation only and all but three invited speakers and participants did in fact join for the workshop. Professors Bob Bronaugh, Richard Guy and James McDougal could not make it for various reasons.

Each participant was asked to present his or her views on a subject that fitted in the major goal of both workshops, which again was to bring together those working on dermal exposure and on dermal penetration, as much as possible related to the daily work of that participant. The main items on the agenda for this two-day workshop are presented in *Appendix 3*.

The specific purposes of the workshop were:
1. To survey and discuss the general state-of-the-science of the methodology for assessing dermal penetration.
2. To identify recommendations for next steps in modeling dermal exposure to consumer products and then prioritize the recommendations for future research.

Further, within Europe greater application is anticipated as a result of the forthcoming Registration, Evaluation and Authorisation of Chemicals (REACH) legislation. It is foreseen that QSARs could reduce greatly the cost of, and number of animals used in, REACH. As a result there is an impetus to provide guidance for the use of predictions from QSARs for regulatory purposes.

PRESENTATIONS

The presentations of all participants (see list of participants in *Appendix 4*) on the first day of the workshop, in so far as they cover scientific content, are summarized by themselves and presented in the following pages. The summaries are presented in the order of the agenda. The oral presentations were followed by a short discussion focused on further clarifications where needed.

DISCUSSIONS AND CONCLUSIONS

On the second day of the workshop (21 June 2005), the discussions took place firstly in break-out groups covering:

1) *Use of Kp in risk assessment (algorithms) and*
2) *Integrating exposure and absorption (how can it be done in modeling approaches?)*

The results of the break-out group discussions were presented by the corresponding rapporteurs in a plenary session and are summarised below. It should be noted that these are group presentations, not necessarily accepted by all participants, as definitely was the case for the general conclusions and recommendations.

A list of research needs as deduced from the workshop discussions is presented at page 17.

It is the intention to produce on the basis of the discussions and result of the workshop a posterior white paper (using as a frame the prior white paper (*Appendix 2*)) that would then be submitted to a known Journal.

DISCUSSIONS OF BREAK-OUT SESSION 1

1. *Use of Kp in risk assessment (algorithms)*

(***Mark Cronin****, rapporteur of break-out session 1*)

The remit of the group was defined as:

- How to use QSAR estimates
- Definition confidence limits on QSAR predictions
- How to use Kp in risk assessment
- How to move to a different dose (concentrations)
- Effect of vehicle
- How to use finite dose
- Inclusion of lag-time with risk assessment to account for exposure period
- Use of tiered approaches – max flux
- How should skin reservoir be handled

How to use QSAR estimates

QSARs may not be accurate enough to deal with small changes in formulation e.g. 1% - 2%. There is a requirement to quantify the accuracy of QSAR predictions. Techniques are available to assign confidence and at the edges of the domain the confidence will be lower than in the center, and outside of the domain uncertainty will be very low.

QSARs may not be able to make predictions beyond an order of magnitude.

Recommendation: Guidance may be required to use a QSAR

Definition confidence limits on QSAR predictions

A tiered approach could be envisaged when the applicability domain is defined, then having the ability to say that it is not possible to make a prediction from a QSAR. Other solvents could be used and QSARs developed for these.

Boundaries of confidence:

- E.g. Potts and Guy; log Kow -1 to 4.

Is octanol-water the best system for partitioning, e.g. membrane-water systems?
To use a QSAR for skin permeation, we should use the variance and co-variance of the original data set.

In current screening guidelines, should maximum flux be used instead of Kp? Max Flux may be more comprehensible for a risk assessor.

Maybe worth giving some worked examples in the different areas of domain of e.g. Potts and Guy to help regulators.

Guidance on how to use QSAR, examples could be given.

Recommendation: How to assign confidence to a prediction.

How to use Kp in risk assessment
Should we use QSARs on maximum flux or use QSARs on Kp and multiply by a value for water solubility (calculated or measured). Water solubility should ideally be measured for chemicals under analysis.

There are difficulties in calculating solubility and melting point.

There may be some experimental data for compounds e.g. solubility, melting point, although prediction methods are available. Many data are available for solubility (e.g. IUPAC).

How do other vehicles alter the skin? Assume maximum flux, this is independent of vehicle (unless the vehicle permeates the skin). However a vehicle may change the flux. Is it possible to make a flux estimation on a vehicle?

A possible concern is the proportion of chemicals within the reliable part of the applicability domain ("happy domain"). If a QSAR will not deal with many chemicals there may be no need to worry about use of Kp as there will be a requirement to measure flux.

Recommendation: Guidance, don't use Kp alone, use with water solubility, more effort on measuring solubility

Effect of vehicle
Accessibility of data in a database for vehicle, and possibly create an algorithm for use by risk assessors.

Formulations are elaborate chemical mixtures designed to target particular parts of an organism.
Volatility of solute / solvent may also be important.

The exposure routes for cosmetics and pesticides need to be considered. They will be different.

Exclusion criteria should be defined for QSARs e.g. if something crystallizes out of a mixture.

Maximum flux is assumed to depend on water solubility in the epidermis. The epidermis is mainly constituted of water, and vehicles that permeate this will alter the solubility characteristics.

Recommendations: Collate and evaluate data, measure data, make predictive models? Identify vehicles where they will alter permeation – where there will be problems for risk assessment e.g. of cosmetics.

Collect data (e.g. literature) for effects of different solvents and affect on permeability, to assess effect of vehicles.

How to use finite dose
Finite dose exposures, how to proceed… Finite dose require 2 parameters e.g. permeability coefficient and partition coefficient. Another QSAR is required for the partition coefficient.

Diffusion coefficients have little variability e.g. for pesticides as there is a narrow molecular weight range, however for consumer products the range may be greater. Sufficient data are required for each chemical to be able to derive fluxes, time of maximum flux, etc., from finite doses, for lipophilic chemicals in particular.

Tiered approach for use of Kp data; within each tier we enter an unknown area where more information may be required.

Use Kp, max flux and, if exposure time is less than 2 times the lag time, use the lag time as exposure time. This will take into account the reservoir effect of the skin.

To convert max flux to an amount transferred corrections need to be made. If the estimated absorbed amount from the maximum flux is higher than the skin load, the absorbed amount should be equal to the skin load (100% absorption).

Uniformity is required amongst exposure assessments, e.g. from EU to US. E.g. Harmonization programmes in US EPA. There is no requirement that programmes change procedures to achieve uniformity.

A method to represent the calculations to obtain information from a finite dose experiment for regulators is required. From the Kp and water-skin partition coefficient the diffusivity is estimated by means of differential equations the finite dose absorption is simulated. Half of the dose will be absorbed at the lag-time.

Recommendation: A method to represent or simplify the calculations to obtain information from a finite dose experiment for regulators is required.

How should skin reservoir be handled?
Should effect of skin reservoir be included? It is accepted in the UK that it should not be included: e.g. stratum corneum. There are concerns however, especially for e.g. hair dyes, for cancer risk assessment. Is it possible to put a figure (e.g. 50%) on how much of reservoir is bioavailable. Hair dyes may be a special case; however, they may not be absorbed. Nevertheless, the inclusion of the skin reservoir in the risk assessment is still a contentious subject.

The EPA Superfund document recommends to risk assessors how to deal with skin reservoir. Can this be used as a template? Overall, skin reservoir should be included with some techniques to determine uptake. Maybe this requires a scenario based consideration. Extending the exposure to 2 x lag-time may eliminate these problems. If flux is low, the absorbed amount will be low: the lag-time is derived from the permeation coefficient and stratum corneum-water partition coefficient as below:

$$lagtime \;\; in finite \; dose \; = \; \frac{\delta^2}{6D} \quad (hours)$$

$$time \; max. absorption \;\; rate \;\; finite \; dose = \; \frac{\delta^2}{6D} \;\; (hours)$$

$$permeation \; coefficient \;\; Kp \; = \; \frac{Psc * D}{\delta} \;\; (cm / hour)$$

$$Psc = 0.64 + 0.25 * Kow^{0.8} \;\; (partition \; coefficient \; SC / water)$$

D = *effective diffusion coefficient in stratum corneum*

δ = *thickness stratum corneum*

Several approaches to deal with the skin reservoir are required, some work is required to evaluate this further e.g. to collate data and investigate them.

There is variability in experimental lag time data.

QSAR for partitioning is required. Some partition coefficient data are available which may be suitable for QSAR modeling.

Examples and guidance on how to compile data may be valuable to regulators. This could lead to higher quality databases. Some guidance may also be required for partition coefficient measurement. Also guidance on how to calculate the value of partition coefficient (see above).

Recommendation: QSAR for partitioning is required. Some partition coefficient data are available which may be suitable for QSAR modeling.

Other comments
Inter-laboratory variability of methyl paraben to study the same membrane. Over an order of magnitude variation, making a saturated solution was amongst the most problematic issues. Maintaining a saturated solution is difficult and possibly a 50% solution would be better, for lipophilic compounds. Revisit literature on effect of solubility.

There is little uniformity in skin preparation e.g. thickness, sources. This may be worth investigating.
For some compounds skin metabolism is important. It may make compounds pass through the skin at a different rate.

Examples and guidance of how to compile data may be valuable to regulators. This could help develop higher quality databases. Some guidance may also be required for partition coefficient measurement. Also guidance on how to calculate the value of the partition coefficient.

DISCUSSIONS OF BREAK-OUT SESSION 2

2. Integrating exposure and absorption. What do we need from the modelers to improve risk assessment?

(***Cees de Heer**, rapporteur of the break-out session 2*)

The need for a detailed look at dermal absorption may both be driven by the degree of exposure to the compound as well as the toxicity of the compound. In the absence of actual data, the extent of dermal absorption can be assessed in a structured way, e.g. by means of a tiered approach as is done for the evaluation of pesticides within Europe. In such a tiered approach, each higher tier brings more refinement into the assessment. Much of the discussion focused on the (further) development of such a tiered approach.

As a first tier, 100% absorption was considered acceptable, although it was envisaged that often a next tier has to be entered. However, if the mass of chemical on the skin cannot be well defined, the maximum flux should be used instead of 100% absorption in tier 1 (two parallel tiers). Examples for the latter are specific exposures situations, such as immersion in a swimming pool and exposure to vapors.

The modeling of dermal absorption could be one of the higher tiers, e.g. by means of PBPK modeling. Since modelers for various reasons have expressed a preference to model the permeation coefficient (Kp) as an estimate for dermal absorption, the question was raised when we can accept a kp for risk assessment purposes? It was recognized that the Kp is not a straightforward measure for finite exposures and that the Kp is vehicle specific. Maybe, however, the Kp can be used to rank absorption at finite exposures. As an alternative, the maximum flux, derived from saturated solutions, may be an alternative product from the mathematical dermal absorption models.

At present, QSARs are available to calculate the flux from an aqueous solution. The solubility ratio can be used to correct the predicted flux values for other solvents. However, this does not take the vehicle effect into account. Therefore, the development of predictive models for vehicle effects was encouraged.

In addition, there was a need expressed for the modeling of absorption from chemical mixtures and formulations.

The following structured tiered approach for the assessment of dermal absorption was developed during the session:

- Tier 1 100% (not for immersion, vapors)
- Tier 2 max flux
- Tier 3a vitro test human or pig skin
- Tier 3b vitro test rat
- Tier 4 vivo test rat (PBPK)
- Tier 5 biomonitoring (PBPK)

In general, mass loading was considered more important than concentration as measure of exposure. With respect to the modeling of (internal) dermal exposure it was concluded that mass loading is usually not evenly distributed over the body (spatial distribution). In addition, regional differences in absorption through the skin are known to occur. This leads to the situation where absorption is

generally overestimated. For these reasons, a probabilistic rather than deterministic approach is preferable for the prediction of (internal) dermal exposure. Such a probabilistic assessment should address both variability and uncertainty of the data, and all stages of the assessment should include realistic input variables (not conservative) to get realistic output. This could include e.g. distributions for species differences, use rate, absorption, and age-related changes in permeability. Unfortunately, present databases do not allow a proper probabilistic exposure assessment (wide confidence limits). For the moment, better default assumptions for a deterministic approach: e.g. based on data like 90% mass loading on 20% of the body area could be a way forward.

The relevance of skin residue dose could be further analyzed based on physicochemical properties. However, this was only touched upon very briefly.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

(***Han van de Sandt***, *chair of concluding session*)

After the presentations of the results of the break-out groups a general discussion took place which led to the following conclusions and recommendations.

1. In occupational situations, the skin contact time is often estimated on the basis of worst case considerations (e.g. 6-8 h per day). However many activities, such as mixing and loading, are generally performed within a much shorter time span. In addition, loading of the skin is not necessarily an instant process, but may occur over time.

2. The deposition of a substance is not homogeneous over the exposed skin area. The variability of the loading of the skin is likely to affect the skin absorption since relative skin absorption (% of dose) of a substance decreases with increasing dose.

3. In order to address points 1 and 2 in the risk assessment, there is a need for probabilistic exposure models. Dedicated studies should provide suitable data for these generic models. New studies may be needed to fill data gaps.

4. From a scientific point of view, the maximum flux should be used in preference to relative absorption in risk assessment. However, it is recognized that this approach may lead to overestimation of the actual skin absorption. QSARs may be used in the following tiered approach:
   - Tier 1 100% absorption
   - Tier 2 QSAR for max flux
   - Tier 3a in vitro testing using human (or pig) skin
   - Tier 3b in vitro testing using rat skin
   - Tier 4 in vivo test in rat (PBPK)
   - Tier 5 biomonitoring (PBPK)

   Guidance on the use of QSARs for regulatory purposes is considered necessary.

5. For further development of QSARs, databases containing measured and well-defined skin absorption data are of great importance. Evaluation of this existing data will allow for proper definition of the use of QSAR (e.g. applicability domain, dose levels, vehicles).

6. There is a need for generating data outside the present applicability domains ("unhappy domain"). Although it is recognized that human in vivo studies are the gold standard, standardized in vitro methodology is considered advantageous for cost-effective testing of substances with toxic or unknown properties.

WORKSHOP-SPECIFIC QUESTIONS AND ANSWERS (with list of research needs)

The specific questions asked to the workshop (see appendix 3) have been answered as follows:

a. How to use JRC EIS Chemrisks "ExpoData" to help drive research needs?

A short presentation on the JRC EIS Chemrisks project was given by the project leader Demosthenes Papameletiou. He indicated the importance of international harmonization of terms and the importance of the approach taken in describing a full database on exposure data. Since none of the participants had had access to the database no further conclusions could be drawn.

b. How to address regulatory policy, specifically EC Guidance Document on Dermal Absorption dated 19 March 2004?

The Guidance document as such has not formed a substantial part of the discussion, apart from the tiered approach as indicated above. The overall approach seems quite reasonable and may be considered a step forward. Specific guidance is also needed for other areas, similar to that developed for the cosmetic area (guidance by SCCP).

c. Considering the tiered approach to modeling dermal exposure, what sort of approach is necessary? A simple approach to screen many chemicals or a refined approach to estimate chemical specific dermal exposure?

It is likely that both approaches may be used in conjunction. The screening approach may hopefully lead to a relatively small number of compounds which need than to be investigated extensively.

d. What are the data needs for modeling dermal exposure to consumer products?

This needs to be dealt with in a more detailed approach such as covered in some of the other workshops in the series. The question has not been answered in the present workshop.

e. Which dermal exposure models are readily available and documented (either separate or integrated)?

This again has been approached in other workshops in the series. The question has not been answered in the present workshop.

f. How to address dermal uptake (experimental and model) uncertainty and variability?

This can best be approached by a second order Monte Carlo approach, forcing more investigations into unknown variables.

g. How to "compare or corroborate" model predictions to experimental results?

Generally more funding should be directed to simultaneous collection of environmental and biomonitoring data in carefully targeted cases. Actual means by which agreement is declared "adequate" are still a matter of research.

h. What are the top priority dermal exposure chemicals/products/scenarios?

This question was not tackled at the workshop.

Listing of clear research needs that were indicated throughout the workshop (not exhaustive)

- *Dermal exposure*
  - Development of techniques that determine mass loading and not mass itself, as a function of time
  - Knowledge on spatial and temporal variation of dermal exposure
- *Percutaneous absorption*
  - Development of QSARs that estimate uptake for relevant conditions (e.g., vehicle, mixtures and finite dose)
  - Relevance of Kow, or another measure, for compounds resembling octanol
  - Experimental and interpretation boundaries pertaining to (specific) QSARs
  - Development of dedicated mechanistic/mathematical models for skin penetration
  - More work is needed on comparison of *in vivo* and *in vitro* methods for assessing skin absorption, using similar experimental conditions
  - Percutaneous absorption from solids (dried liquids) and contaminated soil particles
- *Risk assessment*
  - Relevance of skin reservoir for risk assessment purposes
  - Development of an approach for the use of dermal absorption data other than percentage absorption

WORKSHOP PRESENTATION SUMMARIES – June 20 & 21, 2005

IMPRESSIONS ON THE WORKSHOP ON *'QUANTITATIVE RISK ASSESSMENT'* AT THE OEESC-2005 CONFERENCE IN STOCKHOLM, SWEDEN

Joop J van Hemmen, moderator
Food & Chemical Risk Analysis
TNO Chemistry
Zeist, The Netherlands

Introduction

The Occupational and Environmental Skin Exposure Conference in Stockholm, was the second in a series that started in Washington DC, USA three years ago. The main sponsor and organizer of the series is Sid Soderholm on behalf of the US National Institute of Occupational Safety and Health.

The conference brought together about 200 scientists interested in exposure of chemicals to the skin and the related health effects. At the conference a series of short workshops was organized, one of which was focusing on the relation between exposure and penetration. In a plenary meeting the workshop subject was introduced by Dr. Nick Warren (Health and Safety Laboratory, UK) introducing 'Bayesian and probabilistic dermal exposure modeling', and by Prof. Richard Guy (University of Bath, UK) presenting an overview of 'Method development and modeling to characterize penetration, absorption, dose and local effects resulting from dermal exposures'.

Workshop presentations and discussions

Nick Warren discussed the present state-of-the-art in dermal exposure modeling, where attention is mainly focused on point estimates. By replacing them with distributions, representing variability in work patterns, exposures, personal protective equipment, dermal absorption and other physiological parameters, a probabilistic exposure assessment attempts to characterize the whole distribution of systemic exposure across the work population. 2-Dimensional Monte Carlo simulation can simultaneously evaluate both variability and uncertainty, and thereby, give risk assessors a more scientifically rigorous basis for their decision-making. Modeled uncertainties in systemic exposure can be very large reflecting the cumulative uncertainties in external dermal exposure, mitigation due to clothing or PPE and dermal absorption. In these situations Bayesian techniques that allow the synthesis of dermal exposure measurements with expert judgment and biological monitoring data may provide risk assessors with additional reassurance that margins of safety are met or not. Dr. Warren presented a series of case studies to illustrate the use of these techniques in quantitative chemical risk assessment. No further details will be presented here, since Dr. Warren has extended his presentation for the workshop in Italy.

Richard Guy indicated that from a theoretical standpoint the permeation of chemicals through human skin can be adequately described in most cases by a model based upon transport through the extracellular lipid domains of the stratum corneum, skin's outermost and least permeable layer. Extension of a simple solubility-diffusion model of membrane transport has produced an explicit relationship for a drug's permeability coefficient through the stratum corneum, from an aqueous solution in terms of its molecular size and octanol-water partition coefficient. This, however, pre-supposes a large similarity between octanol and the lipids of the stratum corneum. Although substantial insight into this (dis)similarity has been obtained, additional effort is required to correctly deal with penetration/absorption of very lipophilic compounds and the modeling of non-aqueous vehicles, including particulates (such as soil).

Prof. Guy also described a new approach, called dermatopharmakinetic modeling in which the topical bioavailability by tape-stripping is measured as a surrogate for levels at the target site in the skin. It is clear that this approach is most relevant for pharmaceuticals which are intended to be applied and penetrate through the skin.

Both presentations were widely acclaimed for their transparency and suitability to introduce the main goals for the workshop to the wider audience. The workshop itself was attended by some 70 participants and was further introduced through three short presentations by Derk Brouwer (TNO Chemistry, The Netherlands), introducing 'Spatial and temporal patterns of dermal exposure and the relevance for uptake'. John Cherrie (Institute of Occupational Medicine, UK) discussed 'Dermal exposure and uptake of chemicals for systemic risk assessment. QSARS and other models'. Prof. Annette Bunge (Colorado School of Mines, USA) presented an overview on 'Quantitative risk assessment'.

Dr. Brouwer indicated the importance for using adequate terms in describing the process of exposure. He also expressed the importance of the distribution of material on the skin and its variation throughout the daily work. Realizing these variations indicates the importance of using appropriate measurement techniques that assess the right metric for describing the exposure process and the mass loading onto the skin. Such techniques are not available, although attempts have been made to develop them. The current techniques in use do not estimate the right values for the relevant exposure metrics, such as exposure mass, exposure loading and exposure concentration in all cases.

Dr. Cherrie focused on the results of a recent workshop, on QSAR development and evaluation. Based on statistical analysis the available QSAR models relate the permeability coefficient to properties of the chemicals (QSPeRs). There is, however, not a solid theoretical basis for that. There are further important limitations for industrial chemicals. First of all there are only relevant data for aqueous solutions, and secondly the data only pertain to steady-state conditions (infinite dose). There is quite some development in multi-compartment models which incorporate differences in solubility in different media and predict non-steady state behavior and attempts to cover finite and infinite doses. Research in this area is still emerging.

Prof. Bunge focused in her presentation on the absorbed dose. She shortly described the methodological approaches that are used to estimate the absorbed dose from either the external dose or using QSAR predicted parameters and described some of the difficulties (and possible solutions) encountered with non-aqueous solutions and solids. The major part of the presentation was aimed at the extrapolation from large to small doses. Clear evidence was presented to indicate that the systemically absorbed dose is not independent of the applied dose. The percentage absorption is decreasing when the applied dose goes up. Another issue of concern is the distribution of a chemical over the surface. The dermal absorption is likely to depend on the importance of lateral and transdermal diffusion. Another important issue brought up was the skin reservoir. To what extent should this be taken account of in the risk assessment? Apparently, the current evidence indicates that this may very much depend on the specifics of the compound and needs therefore further research.

The general discussion with some 70 participants in the audience was obviously difficult. The room for the workshop was also rather unsuitable for such a discussion. Nevertheless, John Cherrie, the chairman, stimulated discussions which focused on the set of statements that are included in appendix 1. However, no formal conclusions could be reached with the audience that apparently 'talked

different languages', which was in fact a major reason to bring scientists from different backgrounds together. It proved very difficult, not to say impossible, to understand each other. It proved to be, however, a good first attempt.

The moderator of the workshop, Joop J. van Hemmen, concluded the workshop with the promise that the preliminary white paper, which was published on the website of the conference (at the NIOSH website) and apparently not read by more that one or two members of the audience would be updated after the workshop in Verbania/Intra, where a much smaller audience of scientists dedicated to the subjects would make it in principle much easier to come to conclusions and recommendations for further research.

The final paper would be published on the website and possibly also published as an overview paper in a learned journal.

More details on the OEESC-2005 conference can be found in the Final Programme and Abstracts book, and on the website for the conference.

Acknowledgements
The author acknowledges the support of the Dutch Ministry of Social Affairs and Employment for their financial support of projects dedicated to the subjects of the Stockholm and Verbania/Intra workshops.

References
- Final Programme and abstracts, The Second International Conference 'Occupational and Environmental Exposures of Skin to Chemicals 2005, Karolinska Institute, Stockholm, Sweden (12-15 June 2005).
- Website for the conference: http://www.cdc.gov/niosh/topics/skin/OEESC2/index.html.

SPATIAL AND TEMPORAL VARIATION IN EXPOSURE

Nicholas Warren
Computational Toxicology Section
Health and Safety Laboratory
Buxton, UK

Spatial variation in dermal exposure

The current methodology for dermal exposure assessment is mainly based on assessing total exposure mass. As a result most regulatory risk assessments calculate the systemic dose of a chemical via the dermal route using a % dermal absorption factor. However, the total mass of chemical may not be the most appropriate exposure metric for determining systemic uptake, either because not all the mass is available for uptake or because it is distributed very heterogeneously. A more useful exposure metric might be the contaminated skin area to be used in conjunction with the flux of the chemical across the skin and the duration (or residency time) of dermal exposure. Unfortunately, there is only a weak correlation between the mass of chemical and exposed area (figure 1).

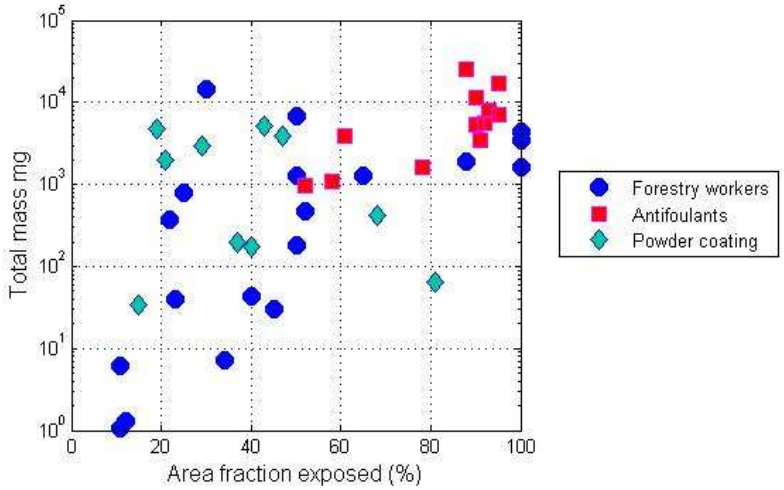


Figure 1. *Correlation between dermal exposure mass and exposed area.*

There is considerable spatial variation in exposure both between individuals and between anatomical regions**.** Figure 2 shows the area fraction that is exposed to a given mass loading for two forestry workers exposed to cypermethrin. For both operations there is a wide variation in the concentration on the overall, but for packers there is a more homogeneous pattern of exposure. The highest exposure concentration for the sprayer is around an order of magnitude higher but 50% of the area is unexposed. In both cases the distribution of exposure across the body is highly skewed with the highest mass loadings covering just a small part of the body. This pattern is repeated across all the spatial data that HSL has collected.

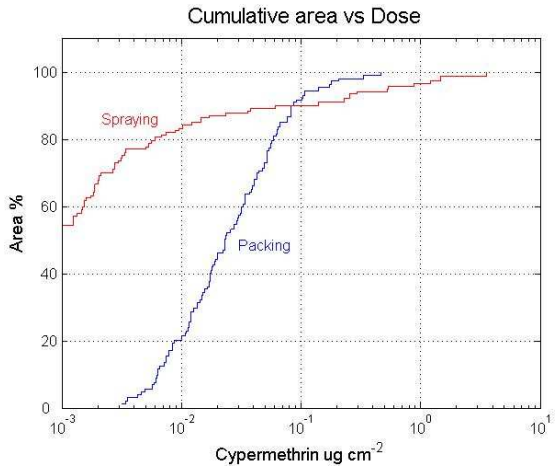


Figure 2. *Area / mass loading profiles for two forestry workers in the UK.*

Overall, around 2/3 of the total exposure is accounted for by the most highly exposed 10% of the body surface area (figure 3) - although the location of this area will differ between individuals. This may have an important influence on dermal absorption –with most of the dermal exposure occurring at considerably higher mass loadings than the mean mass loading over the entire body. If the absorbed dose per unit area is not proportional to the applied dose then a default assumption of uniformly distributed exposure will result in an over-estimate of the systemically absorbed dose.

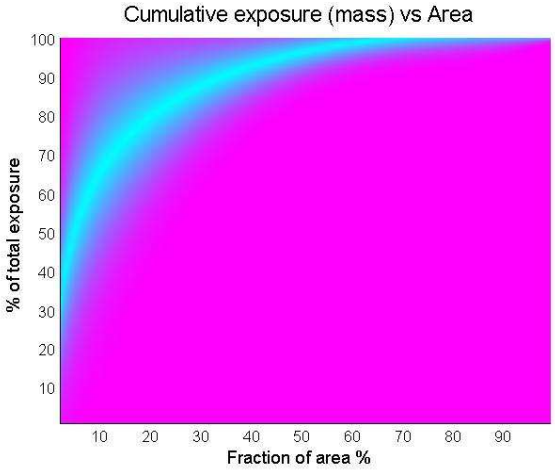


Figure 3. *Variation in the mass loading profiles between individuals.*

The effect of spatial heterogeneity in exposure on absorption has been examined using a case study for lindane (where in vitro data shows % dermal absorption is dose dependent – Zendzian 2000). This case study compares estimates of systemic dose based upon mean mass loadings with estimates calculated from the entire distribution of mass loading for 41 workers hypothetically exposed to lindane. Mass loading profiles were taken from previously monitored workers using the Dirichelet–PXRF technique (Wheeler 2002). Assuming a spatially uniform pattern of exposure has been shown to overestimate systemic dose by up to 2.5 times (figure 4).

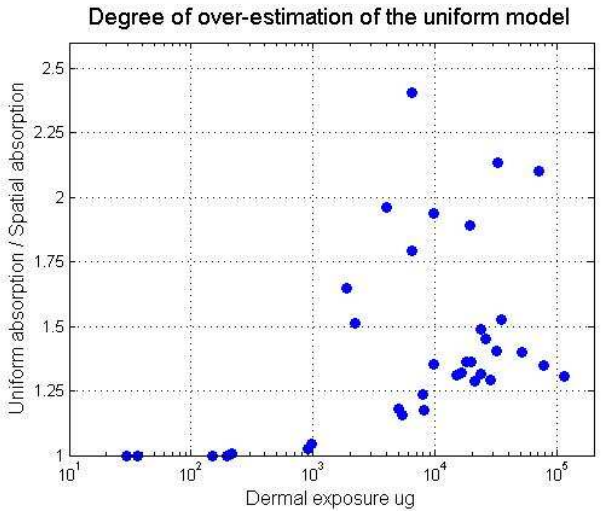


Figure 4. *Degree of over-estimation attributable to the assumption of homogeneous dermal exposure.*

One approach would be to make a calculation based upon an assumption of spatially uniform dermal exposure and then apply an uncertainty factor to represent the unknown effect of spatial variation. The distribution of this factor could be obtained from a similar analysis to that shown in figure 4.

The alternative method of calculating systemic dermal dose based upon flux and the exposed surface area is critically dependent upon realistic estimates of the skin area covered by the chemical. The estimation of this area, particularly at low levels of exposure (such as pesticide residues), is problematic with estimates varying depending upon the spatial resolution of dermal assessment method

Temporal aspects of exposure and modeling dermal exposure over multiple days

Traditionally, occupational exposure assessments have tended to focus on determining systemic exposures resulting from a single exposure scenario, work-shift or day. Longitudinal modeling considers the profile of systemic exposure over a longer period – perhaps weeks, months or even years. Such an approach has several advantages. For chronic health-effects cumulative exposure (or equivalently average exposure over the relevant time period) provides a more appropriate exposure metric than a short-term daily dose and allows risk assessments to be based upon the probability of long-term over-exposure. Additionally, uptake of a chemical following dermal exposure can continue over a number of days so that systemic exposure in a 24-hour period is a composite function of the previous day's exposures. These 'residual' contributions to systemic exposure are not captured by single-day assessments. In these circumstances, systemic dose on a given day is a composite of contributions from the current and several previous days. It is possible to model such systemic exposure using a moving average process:

$$Systemic_i = \ldots + \%\, abs_{48-72} \times ADE_{i-2} + \%\, abs_{24-48} \times ADE_{i-1} + \%\, abs_{0-24} \times ADE_i$$

Where ADE_i is the actual dermal exposure on the i^{th} (current) day, ADE_{i-1} the actual dermal exposure on the previous day, % abs $_{0-24}$ is the % dermal exposure occurring in the first 24 hours after exposure, % abs $_{24-48}$ is the % dermal exposure occurring in the 2nd 24 hours after exposure etc. Represented in this manner, systemic exposures over multi-day periods (longitudinal exposure modeling) can be evaluated using probabilistic techniques. Proper consideration of the absorption process over multiple days can lead to a smoothing of the predicted uptake of a chemical via the dermal route and a corresponding reduction in intra-individual variation in exposure. In turn, this has implications for risk assessment as regulatory risk assessments are usually based upon high-end exposure percentiles.

References

- Wheeler J P and Warren N D (2002). A Dirichlet Tessellation-based Sampling Scheme for Measuring Whole-body Exposure. Ann Occup. Hygiene Vol. 46, No. 2, pp 209-217.
- Zendzian, R P (2000). Dermal absorption of pesticides in the rat. Am. Ind. Hyg. Assoc. J. 61, 473-483.

A USERS EXPERIENCE WITH THE DERMAL MODULES OF CONSEXPO AND PROMISE, AND A VISION FOR FUTURE MODELS

Tipton Tyler
Health Studies Management & Consulting
4212 Wynnwood Drive, Annandale, VA 22003 USA

Abstract
The dermal uptake of a hydrocarbon solvent from an auto polish scenario was used to evaluate two scenario-based models useful in estimating exposure to consumer products; n-decane, a component of the hydrocarbon solvent, was evaluated for purposes of this study. Both models proved useful for the estimation of dermal exposure. Modules using skin permeability estimation procedures that were common to both models gave similar but not identical results for the systemic uptake. A 2-fold difference was found in doses obtained using estimation procedure giving the lowest and highest rates of skin permeation. A much lower dermal uptake, some 3 to 4 magnitudes lower than with the estimating procedures, was obtained when experimentally derived skin permeability or flux values were used. A comparison of a number of features between the two models and some suggestions for improvement, particularly with respect to probabilistic modeling are given.

Introduction

This study was conducted to better understand the capabilities of two different computer simulation programs, PROMISE version 7.0 (Sielken, R. L. (1998) and ConsExpo version 4.0 (Van Veen, M. P., 2001), used in scenario-based chemical exposure modeling. This report addresses the modules of these two programs used to assess dermal uptake into the systemic circulation. The exposure scenario used in this study was that of a teenager using an auto polish, and is described as follows:

> "A teenage male weighing 70 kg polishes a car in a garage. The polish is a slightly viscous fluid consisting of water, hydrocarbon solvents, and various polishing and emulsifying agents with a specific gravity of 0.95. Approximately 119 ml was applied to the car surface. The operation consisted of applying the liquid polish to the auto surface and then, after drying, the surface was buffed with a clean, dry cloth. The polish was applied by pouring the solution onto a cotton cloth and then rubbing that onto the auto finish with an ungloved hand. The entire operation required 30 minutes, half of which was involved in applying the polish (15 minutes), the remainder in buffing the surface."

Methods

For both models, a contact surface area of 228 cm^2 was assumed. This is equivalent to the skin area of the palm of an adult hand (ICRP, 1975). A skin contact time of 0.33 minutes (20 seconds) was used. This value was based on the assumption that it took 5 seconds to pour polish onto the cloth and 15 seconds to rub the polish on the auto finish. It then follows that 45 of these events occurred in the 15-minute polish application period. Based on the assumption that 10% of the polish applied to the cloth came in contact with skin, the contact volume was calculated to be 0.26cm^3 (11.9cm^3/45 applications = 0.26). The concentration of n-decane in the polish was assumed to be the entire weight content of the solvent and was calculated to be 427 mg/cm^3 (119cm^3 X 0.95g/cm^3 = 113g X 45% = 50.85g n-decane/119cm^3 = 427mg/cm^3 auto polish). The dermal uptake of n-decane was calculated either by using one of a number of equations to estimate skin permeability or by using an experimentally derived dermal flux for decane, 1.65 ug/cm^2/hr, or the skin permeability coefficient,

5.5 X 10^{-5} cm/hr (McDougal, J. N, et al., 2000). The experimental values were obtained from *in vitro* studies with human skin using static diffusion cell apparatus.

The equations used to estimate skin permeability are generally based on the molecular weight and octanol water partition coefficient of the material under study. For n-decane a molecular weight of 140 along with the log $Kp_{(oct/H20)}$ of decane,6.25, was used (McDougal, J. N. et al., 2000). ConsExpo allows the user to directly input a skin permeability value whereas PROMISE requires some type of manipulation to use experimentally derived values. For PROMISE the flux value was used to determine the total quantity of n-decane penetrating the skin in 20 seconds and total uptake determined by using this quantity, 100% fractional uptake and 45 events per day.

Results

The values for the absorbed dose of n-decane obtained using both models and the various skin permeation estimating equations or using the experimental derived skin permeability approaches are given in Table 1. Both PROMISE and ConsExpo version 4.0 use three of the same skin permeability estimating equations. In general these estimating procedures gave fairly consistent results with only an approximate 2 fold difference between that giving the lowest estimate, McKone & Howd using PROMISE and those giving the highest estimates Fiserova-Bergerova, Guy & Potts and the Bogan equations using ConsExpo. Both models gave similar dose estimates using the Fiserova-Bergova and Guy & Potts procedures, 71.2 for PROMISE and 72.3 mg/kg/event for ConsExpo. These values represent essentially complete absorption, as does the value obtained by ConsExpo using the Bogan equation. A slight difference was obtained between models when using the McKone & Howd equation. The reason for this difference is not clear but it should be noted that PROMISE asks for the explicit input of blood volume and flow rates at the site of contact. ConsExpo does not ask explicitly for these values, but instead uses default values that might be somewhat different from the default values of PROMISE and used in this study[1]. In addition it is possible that these models might use different calculation routines that account for the difference.

Of greater significance, however, were the extremely large differences between the absorbed doses calculated by the various permeability estimating routines and doses obtained using experimentally derived values of skin permeability or flux. The doses calculated from these experimentally derived rates were three to four magnitudes lower than those obtained by the estimating procedures. Again, the reason for these large discrepancies is not fully understood but may relate to the fact that the estimating procedures are based on the permeation of chemicals from aqueous solutions. The alkanes that are used in the auto polish are relatively water insoluble and the experimentally derived permeation rate (flux) used was obtained using "neat" (undiluted) decane. Thus, the fact that the flux value was not obtained from an aqueous solution may account for some of the discrepancy between the experimentally derived result and that using the estimation procedures.

Discussion

The main objective of this study was to gain experience with the two models used for scenario-based exposure assessment. Both these models are particularly useful in assessing exposure to consumer products. The work reported here was, in fact, part of a larger study that not only investigated exposure due to dermal uptake, but also to uptake from vapor inhalation. Therefore many of the observations regarding model attributes and needs address both these routes. ConsExpo version 4 is clearly much more "user-friendly" than PROMISE. On the other hand, PROMISE provides more interim data, i.e. tabulated percentile output and mass balance information, and its calculation

[1] Personal communication with Christiaan Delmaar, RIVM, Bilthoven, The Netherlands

algorithms appear to function somewhat faster than those of ConsExpo. This latter point is evident when conducting probabilistic analyses for which both models have capability. One of the really convenient features of ConsExpo is the ability to enter a large variety of units for most input values, a feature not found in PROMISE.

Table 1. *Dermal absorption of n-decane obtained using two different scenario-based exposure models, different skin permeation estimating equations or experimentally derived values*

Procedure	**Dose (mg/kg/event)**	
	PROMISE	**ConsExpo**
Fiserova-Bergerova	71.2	72.3
Guy & Potts	71.2	72.3
McKone & Howd	37.6	58.4
Robinson	66.6	
New Robinson	42.3	
TenBerge		54.1
Bogen		72.3
Modeled using Flux Rate (Promise)	0.001	
Modeled using Skin Permeability Coefficient (ConsExpo)		0.04

PROMISE does have great versatility in allowing for input of variables necessary in the calculation of results. It is, however, difficult to obtain meaningful data on many of these variables, for instance blood volume and blood flow rates at site of contact, and the user generally ends up using default values. ConsExpo simplifies the problem for many of these difficult to obtain values by simply using defaults. It should be noted that PROMISE does have a linked library with referenced input values that can be selected by users. PROMISE would certainly benefit from an ability to directly enter experimentally derived skin permeation or flux values.

A major deficiency of both models is their inability to take into account dependency of input variables when conducting probabilistic analyses. For instance, when independently varying the body weight and skin contact area of an individual, it is likely that some simulations will use very small contact areas with very large body weights and vice versa. Similarly, in painting scenario it is likely that some simulations will use unrealistically long application times with very small room sizes. This is a difficult problem and this author is not aware of any scenario-based exposure models that address it successfully. The problem can be alleviated to some extent by careful thought given to the input values with an attempt to avoid dependency. Thus ConsExpo avoids the room size application dependency by using a rate of application input variable.

In lieu of resolving the dependence problem, however, a tabulation of each input variable for each simulation would be helpful such that the user could examine the inputs of the upper end percentiles to evaluate their relevance to real world situations. Neither ConsExpo nor PROMISE incorporates this feature in their current design.

Conclusions

The dermal uptake of n-decane from an auto polish scenario was used to obtain experience with the dermal modules of two consumer oriented, scenario-based exposure models, ConsExpo and

PROMISE. Seven different skin permeability estimating procedures were used between the two models, three of them common to both. The estimating procedures common to both models yielded relatively consistent but not identical results. Overall there was an approximate two-fold difference in dose between skin permeability estimating procedures yielding the highest and lowest values. A much greater difference in dose, three to four magnitudes lower than that used in the estimation of skin permeability, was obtained when using experimentally derived values for skin permeation or flux.

Both models proved useful in estimating exposure in the auto polish scenario. ConsExpo was much easier to use whereas more data output was provided by PROMISE. Dependency of input variables is a problem shared by both models and probably by all the current scenario-based models capable of probabilistic approaches. Careful thought to avoiding dependent input variables and the output of input variables for each simulation would be useful modifications in these models.

Acknowledgement
The author is greatly indebted to the American Solvents Council who, in part, sponsored this work.

References
- Sielken, R. L. (1998) Probabilistic Methodology for Improving Solvent Exposure Assessment (PROMISE). Prepared for the Solvents Council of the Chemical Manufactures Association. Downloadable version available on the American Solvents Council Web site: http://www.americansolventscouncil.org/resources/promise.asp.
- Van Veen, M. P. (2001) CONSEXPO Version 3.0. RIVM - National Institute for Public Health and Environmental Protection. Bilthoven, The Netherlands.
- ICRP (International Commission on Radiological Protection) (1975) *Report of the Task Group on Reference Man* No. 23. "Chapter 1. Anatomical Values for Reference Man." Pergamon Press, Oxford.
- McDougal, J. N., Pollard, D.L., Weisman, W., Garrett, C. M. and T. E. Miller (2000) "Assessment of Skin Absorption and Penetration of JP-8 Jet Fuel and Its Components" Toxicol. Sci. 55:247-255.

EFFORTS TO HARMONIZE DERMAL EXPOSURE METHODS AT EPA

Michael Dellarco
US EPA, National Center for Environmental Assessment
Washington, DC, USA

Dermal exposure to chemical contaminants occurs via exposure to water, soil, and air, direct contact with treated surfaces or pure chemicals, frequently in mixtures. The site of dermal exposure is directly related to the activity being performed at the time of exposure and depending on the media and anatomical site of contact, the contaminants may be absorbed differently. Several factors can influence dermal exposure (Kissel, 1996; Dermal Exposure Network, 1999). These include:

- Reduction or increases in the chemical contact with skin due to clothing;
- Protective clothing and gloves and the amount of protection they offer;
- Individual differences in dermal exposure due to differing degrees of speed, care, and dexterity in performing work;
- Variance in the amount of material available for dermal absorption due to actions such as wiping the affected area with the hand;
- Variances in the penetrability of the skin in different parts of the body;
- Individual variability in regard to skin penetrability due to age and skin condition, such as thickness of the stratum corneum; and
- The matrix of the chemical contaminant, solid, liquid or vapor.

The amount of chemical coverage on the skin surface can influence the amount of dermal absorption. Chemical coverage of the skin may be incomplete or exceed the exposed skin surface area by piling up. Likewise the transfer efficiency from a contaminated surface or liquid solution to the skin may be highly variable due to the nature and extent of the contact, chemical composition, or the deposition of chemical residue due to evaporation of the liquid. Passive diffusion is considered to be the main processes of dermal penetration of chemicals through the stratum corneum. After a chemical has passed through the stratum corneum, the outermost layer of the skin, it can be transferred through the viable epidermis (the next skin layer) into the dermal blood supply and on to the systemic circulation. Dermal penetration can be measured by *in vivo* or *in vitro* procedures.

In vivo techniques can be used to measure dermal penetration either directly or indirectly (Bunge and McDougal, 1999). In indirect techniques dermal absorption is inferred from the surface disappearance of the chemical. In direct methods chemical is measured in the blood or excreta, on strips of tape that progressively remove stratum corneum or implied by biological or pharmacological responses. The following list describes several *in vivo* methods used to estimate dermal absorption (Wester and Maibach, 1999):

- Surface recovery. The amount of chemical remaining at the end of the exposure is measured (i.e., the recovered dose). The absorbed dose is assumed to be the difference in the applied dose and the recovered dose.
- Surface disappearance. The disappearance a radiolabeled compound from the surface of the skin is measured on the skin (i.e., the chemical is not removed) using the appropriate instrumentation. This method is limited because the techniques used do not measure chemical that has absorbed into the skin.

- Measuring the total amount of chemical appearing in the excreta. The compound (often radiolabeled) is applied to the skin and the total amount of excreted in the feces and urine (i.e., measurements continue until the concentration is below detectability) is compared to the amount of excreted following a parenteral administration. When determined by radioactivity, this method does not account for dermal or systemic metabolism because the amount of radioactivity would include both parent compound and metabolites.
- Measuring the total amount of chemical in the blood. This is measured by the ratio of the areas under the plasma concentration versus time curves following dermal and intravenous administration. When radiolabeled chemicals are used, this method does not account for dermal or systemic metabolism because the radioactivity could include both parent compound and metabolites (unless combined with methods separating parent and metabolite).
- Biological and pharmacological response. A biological assay is substituted for a chemical assay. Absorption is estimated from observing the magnitude of the biological response. This method is limited because compounds must elicit responses that can be measured easily.
- Tape stripping method. This method determines the concentration of the chemical in the stratum corneum after a specified exposure time. The technique involves sequentially applying adhesive tape strips to the exposed site (after the remaining surface chemical is removed) until all of the stratum corneum is removed.

Some *in vivo* procedures measure percutaneous penetration. For example, the *in vivo* protocol specified by the US EPA for testing pesticides measures the amount in excreted material during the exposure and the amount in the carcass at the end of the exposure (Zendzian, 1994; 2000). In addition, the amount in the washed skin from the exposed site is determined. Provided that the wash is 100% efficient, this combined with the amount in the carcass and the excreted material should be the total amount dermally absorbed.

Indirect *in vivo* techniques have been used successfully but there are some drawbacks. These techniques can be used only for chemicals that are not volatile. Direct *in vivo* testing is more complicated and time consuming; however, they can provide estimates of the total absorbed amount of chemical in the blood or tissue and the amount eliminated (Zendzian, 2000). Pharmacokinetic modeling can also be used to estimate absorption from blood, exhaled breath, or tissue concentrations (Bunge and McDougal, 1999). The tape stripping method can be used to determine the amount of chemical in the stratum corneum. However, disadvantages of the tape stripping method includes, the stratum corneum must be stripped completely and rapidly, chemical analysis can be difficult because the amount of chemical can be small, and there can be a large amount of data variability due to irregular skin stripping efficiency.

In vitro methods have appeal because they lack use of live animals, are less expensive than in vivo methods, can be used with skin from several species, including humans, and can asses the impact *in vitro* procedures can be used to estimate dermal absorption. However, he did not use appropriate statistical procedures to make these comparisons (Sartorelli *et al.*, 2000) of chemical toxicity or skin damage without ethical issues. Two different types of *in vitro* techniques have been used to study dermal absorption, the infinite dose and finite dose technique (OECD, 2000; Sartorelli *et al.*, 2000). The infinite dose technique is the most frequently utilized method. It involves mounting the skin as a barrier between two chambers of fluid. A large amount of chemical, usually in water, is added on one side and absorption is quantified by measuring the concentration in the receptor solution on the other side as a function of time. Measurements are continued until steady state is achieved as indicated by or the cumulative mass in the receiving chamber increasing proportion to time. The permeability

coefficient is then calculated using the slope of the linear regression of the cumulative mass versus time (Bunge and McDougal, 1999). In the finite dose technique skin is mounted in a diffusion cell and bathed from below by isotonic saline kept at a temperature of 37°C. The donor chamber contains a known amount of the chemical and the concentration of the penetrating chemical is measured in the receiving chamber to provide a measure of the cumulative amount that has penetrated a specified area of skin in a given exposure time. The advantage of the finite dose technique is that it allows for any type or amount of substance to be tested in conditions similar to the living state. The chief disadvantage is that meaningful permeability coefficients cannot be determined.

One of the major factors affecting *in vitro* percutaneous penetration results is the choice of receptor fluid for collecting the chemical that penetrates the skin. Generally, it should provide sink conditions without altering the skin barrier function. The current OECD guidelines require that sink conditions be insured by proving adequate solubility in the receptor fluid (OECD, 2000). The receptor fluid should be chosen to maintain skin metabolic activity when fresh skin is used and the absorbing chemicals may be metabolized.

Efforts to compare *in vivo* and *in vitro* dermal absorption methods have generated mixed results (Franz, 1975; Dellarco *et al.*, 2000; Zendzian and Dellarco, 2002). *In vitro* methods may overestimate or underestimate *in vivo* measurements depending on the chemicals involved, the experimental procedures followed and the data analysis procedures used. *In vivo* measurements for exposure times that are not long relative to the lag time will normally overestimate the steady-state permeability coefficient because dermal absorption is initially faster than at steady state. Bunge and McDougal (2000) concluded that this is consistent with the "widely stated observation that *in vivo* permeability coefficients are larger than those measured *in vitro*". This may not reflect differences in *in vitro* and *in vivo* testing methodology, but errors in data interpretation (Bunge and McDougal, 1999). Notably, *in vivo* measurements that determine penetration can underestimate the steady-state permeability coefficient unless the lag time is considered in the data analysis. Franz (1975) compared results from *in vivo* and *in vitro* tests and concluded that compared *in vivo* and *in vitro* dermal absorption methods in different species. They found the *in vivo* results for lag time, maximal flux and systemically available amount varied considerably between rat and human. All results from *in vitro* methods were similar to human *in vivo* methods based on absorbed dose. However, maximal flux and amount systemically available were significantly overestimated for the human *in vivo* model using *in vitro* methods. Zendzian and Dellarco (2002) compared *in vivo* and *in vitro* dermal absorption data in the rat for acetochlor and found that the results from the *in vitro* method did not approximate those obtained for the *in vivo* method. The Percutaneous Penetration Subgroup (PPS) of the Dermal Exposure Network (DEN) published a report that focused on standardization and validation of *in vitro* experiments (Sartorelli *et al.*, 2000). The objectives of the PPS were to analyze the guidelines on percutaneous penetration *in vitro* studies presented by various organizations and suggest standardized *in vitro* methods while taking into account their individual research experience, literature data and existing guidelines. Key issues and data gaps reported included:

- How to use percutaneous penetration data in risk assessment;
- Factors influencing the results from percutaneous penetration *in vitro* studies (i.e. the choice of the donor phase, cell characteristics, skin membranes present, and receptor fluids);
- Agreement on and validation of existing guidelines for conducting *in vitro* studies;
- Use of penetration data to predict plasma levels;
- Effects of cutaneous metabolism on dermal absorption;
- The selection of appropriate reference chemicals for *in vitro* study;

- Use of microdialysis in *in vivo* studies; and
- The correlation of *in vitro* and *in vivo* study results.

Recently, the US EPA's Risk Assessment Forum conducted an evaluation of dermal absorption methods used in the Agency as part of an effort to harmonize dermal exposure assessment procedures (US EPA, 2005). They found that harmonization was impeded by method differences (in vitro vs. in vivo methods) and procedures used to estimate dermal transfer efficiencies. More study is required to evaluate the comparability of *in vivo* and *in vitro* dermal absorption procedures.

References

- Bunge AL. 2000. Reducing uncertainty in dermal exposure assessment by developing quantitative methods for animal-to-human extrapolation. Proposal.
- Bunge AL, McDougal NJ. 1999. Dermal uptake. In: Exposure to Contaminants in Drinking Water. Estimating Uptake Through the Skin and by Inhalation. Olin SS, ed. CRC Press, Boca Raton, Florida. Pp. 137-181.
- Dellarco M, Zendzian RP, Cardello N. 2000. Determining the validity of in vitro dermal penetration studies to estimate percutaneous absorption of chemicals. Presented at the Annual Meeting of the Society for Risk Analysis, Arlington, VA, December 3-6, 2000.
- DEN. 1999. Issues Relating to the Risk Assessment of Dermal Exposures. University of Surrey. December.
- Kissel JC, Richter KY, Fenske RA. 1996. Field measurements of dermal soil loadings attributable to various activities: implications for exposure assessment. Risk Anal 16(1):115-125.
- Franz, TJ. 1975. Percutaneous absorption: on the relevance of in vitro data. J. Invest. Dermatol. 64:190-195.
- OECD. 2000. OECD Guideline for the Testing of Chemicals. Draft New Guidelines 428: Skin Absorption: *in vitro* Method Draft. December 2000.
- Sartorelli P, Andersen HR, Angerer J, Corish J, Drexler H, Goen T, Griffin P, Hotchkiss SAM, Larese F, Montomoli L, Perkins J, Schmelz M, van de Sandt J, and Williams F. 2000. Percutaneous penetration studies for risk assessment. Environ Toxicol Pharmacol. 8:133-152.
- Wester RC and Maibach HI. 1999. *In vivo* Methods for Percutaneous Absorption Measurements. In: Percutaneous Absorption. Third Edition, Revised and Expanded. Bronaugh RL, Maibach HI, eds. Marcel Dekker, Inc., New York.
- Zendzian RP. 1994. Pesticide Assessment Guidelines. Subdivision F: Hazard Evaluation, Humans and Domestic Animals. Series 85-3. EPA.
- Zendzian RP. 2000. Use of in vitro dermal penetration studies to compare rat and human penetration of chemicals. Presented at the Annual Meeting of the Society for Risk Analysis, Arlington, VA, December 3-6, 2000.
- US EPA. 2005. Dermal exposure assessment: A summary of EPA approaches. January 7, 2005.

DERMAL ABSORPTION DATA GENERATED BY IN VITRO METHODS*:* WHY DO THEY DIFFER FROM QSAR PREDICTIONS?

Faith M Williams
University of Newcastle, Newcastle, UK

A range of approaches have been used to define percutaneous penetration of chemicals including studies in animals following application *in vivo*, *in vitro* absorption studies with rodent skin or with human skin and studies in human volunteers in which the chemical is applied to the skin and the internal dose monitored. The *in vivo* data can be used to validate the *in vitro* studies. Percutaneous penetration data can also be obtained from modeling approaches such as QSAR (quantitative structures activity relationships) and computational models. All of these approaches are required to generate new exposure relevant dermal absorption data that can be used in the risk assessment exposure to chemicals Evaluation of the different approaches is particularly important currently with the EU requirements for generation of risk assessment of chemicals under the REACH Regulations. There has been particular emphasis on the use of *in vitro* methods with isolated human skin, pig skin, or rodent skin, in order to reduce the use of animals for toxicology within the European Union and a number of guidelines and protocols have been established for conducting these studies (OECD etc). The European Commission has funded a multi centre research project (EDETOX www.ncl.ac.uk/edetox, Williams et al., 2004) to assess a range of approaches to obtaining directly occupationally relevant dermal absorption data. Within this project absorption data was generated using a protocol which followed the OECD guidelines fairly closely but allowed the flexibility inherent in the guidelines between laboratories (Sandt et al., 2004). The robustness of the *in vitro* method was assessed between 10 laboratories using standardized application of model substrates, benzoic acid, testosterone and caffeine, but a range of cell designs, flow through and static and full thickness and dermatomed human skin. The choice of receptor fluid ensured that the absorbed material was soluble. This study allowed an assessment of the inter-laboratory variability and the inter-skin variability Inter-laboratory variation was great than intra laboratory variation although ranking was the same for all laboratories. There was an influence of the thickness of the skin used for the absorption studies, particularly for lipophilic testosterone where full thickness human skin resulted in significantly lower absorption to receptor fluid than dermatomed skin. The study also found that variability was introduced by use of different samples of human skin both within laboratory and between laboratory. The variability between samples of skin was greater than the variability between cells using the same sample of skin within a laboratory. Variability between the permeability of different human skin samples contributes to difficulties in standardizing the technique between laboratories, where it was not possible to control the samples of skin to be used and the numbers were, by necessity, only small because of skin availability. When considering the influence of full thickness versus dermatomed human skin in the flow through system there was a five-fold difference in the flux and in the total amount absorbed by 24 hours for testosterone but a smaller effect for caffeine of 50% increase. (Wilkinson et al., 2005) It was previously shown that testosterone absorption (both flux and total amount absorbed) varied eight-fold between eight samples of human female breast skin (Lee et al., 2001).

The variability identified between human skins (Sandt et al 2004) and that with a silicone membrane (Chilcott et al., 2005) have stimulated discussion about the need to tightening the guidelines. This was particularly addressed at the CEFIC Workshop (Jones et al., 2004 www.iom.org) where protocols were proposed for generating data using both finite and infinite doses that might be used to

support the application of data derived from existing QSAR models to obtain exposure relevant absorption data.

When conducting dermal absorption studies two approaches can be used; firstly to obtain exposure relevant data generally by applying a finite (small) dose in the appropriate vehicle to simulate drop exposure and allow evaporation. Secondly to generate data relevant to the QSAR approaches with the application of an infinite dose of a saturated solution in water. (An infinite dose application results in no significant changes in volume and concentration of the dose during the experiment). The absorption profiles obtained may differ significantly. Following an application for an infinite dose, steady state absorption is established and continues throughout the whole study, whereas for a finite dose steady state absorption may be established for a short time and then with loss of material from the surface of the skin, either by evaporation or by absorption, the rate will decrease or because of these factors a steady state absorption profile will never be obtained. This needs to be taken into account when applying data derived from Kp from a QSAR to finite dose exposure situations. It is important to have selected the appropriate dose and vehicle in a finite dose to establish relevance to the actual exposure situation. The distribution profile through the skin is an important determinant of the relevant interpretation of the data; in particular the amount of material remaining in the stratum corneum reservoir should be determined in order to make a decision about whether this should be included as absorbed, potentially available for absorption, or not available for absorption. The approach is important for lipophilic molecules where the stratum corneum reservoir may be significant. The question of availability of this lipid bound material has entertained considerable discussion and for lipophilic pesticides the current guideline is that this material should not be included as absorbed or potentially available.

Results obtained during the EDETOX project do not indicate that model predictions can substitute for well designed *in vitro* absorption studies when there is a requirement to generate relevant data for risk assessment. Infinite dose data was generated for 21 chemicals that were not currently included in the Flynn or Patel database, and not used in the current relationships described by Potts and Guy (Fitzpatrick, Corish and Hayes, 2004). The infinite dose data generated for these 21 chemicals fitted well within the domain between log p 0 and 4 and obeyed the relationship described by the Potts and Guy equation.

For some chemicals applied as a finite dose the flux observed was very much over estimated by the flux calculated using the Kp derived with an infinite dose. Therefore, not only was the flux misleading but also the absolute amount absorbed and percentage dose absorbed tended to be an overestimate. In comparative studies an infinite dose of 90% saturation in water (200µl in flow through) was compared with a finite dose, also at 90% saturation in water (10µl). The maximum flux measured at finite volume was significantly lower than from an infinite dose though the rank order was similar for malathion testosterone, parathion and triclosan. Following the finite dose application the phase of maximum flux was short though absorption continued throughout the time course to some extent, even after evaporation of the vehicle, the apparent lag time of the finite dose was reduced compared with the infinite dose. There was no absolute relationship between the dose absorbed under the finite dose conditions and log p although similar proportions of malathion and testosterone were absorbed in 24 hours despite the difference in log p (Wilkinson et al 2005).

The effect of vehicle was determined by application of solute in different vehicles at equivalent saturation when thermodynamic activities were similar. For caffeine the flux related to the concentration in the different vehicles and was independent of the nature of the vehicle. The greatest

flux was from a 50/50 water/ butoxyethanol mixture where 25% saturation was 10mg/ml, compared to water 25% saturation at 4.4mg/ml and butoxyethanol 25% saturation 2.25mg/ml. and the Kp values were similar (Traynor et al 2005). However, for testosterone (log p 3.4) 25% saturated testosterone in butoxyethanol at 29mg/ml had a similar flux to that at 25% saturation in 50/50 butoxyethanol /water at 5mg/ml and the Kp from butoxyethanol /water was six times greater than that from butoxyethanol. Similar results were obtained for 50% saturated solutions in butoxyethanol and butoxyethanol/ water and octanol.

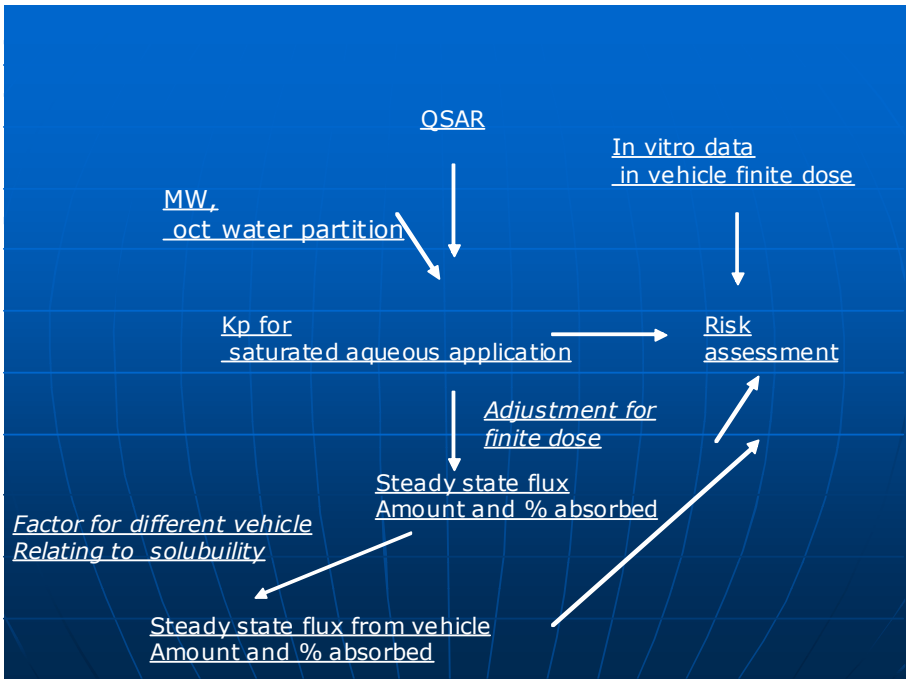
An apparent interaction occurs between butozyethanol and water and with the skin barrier resulting in changes in absorption from the mixtures although the mechanism is not fully understood. Butoxyethanol absorption from a water mixture was very much greater than from a neat solution (Wilkinson et al., 2004) and the Kp increased indicating an interaction of the butoxyethanol/ water mixture with the skin resulting in barrier changes and deviation from Fick's law of Diffusion. This was confirmed by comparing absorption through a silicone membrane where there was a fixed relationship between concentration and flux as the butoxyethanol was diluted in water. (Traynor et al., 2005) This effect of dilution of butoxyethanol in water on absorption through skin was also observed *in vivo* in human volunteers (Jakasa et al., 2004) and in rodents.

In conclusion, in vitro absorption studies using human skin conducted in line with the Guidelines provide exposure relevant absorption information. Flux and amount absorbed derived from QSARs which predict Kp values currently has limitations because of a tendency to overestimate the actual absorption when extrapolating from the saturated aqueous solution to the actual vehicle giving a worst case scenario. A major limitation is the lack of information on the influence of vehicles on the absorption of solute through skin. This information is required before QSAR predictions from aqueous databases can be applied. and it is important to generate data using a range of vehicles of different physicochemical properties and their influence on absorption of a series of marker chemicals eg caffeine, and testosterone in order to generate some rules which could be used for applying QSAR Kp derived absorption fluxes for risk assessment . A decision making process could be established to predict whether a vehicle would influence absorption by interaction with the skin (see figure)

References

- Chilcott RP, Barai N, Beezer AE, Brain SI, Brown MB, Bunge AL, Burgess S, Cross S, Dalton CH, Dias M, Farinha A, Finnin BC, Gallagher SJ, Green DM, Gunt H, Gwyther RL, Heard CM, Jarvis CA, Kamiyama F, Kasting GB, Ley EE, Lim ST, McNaughton GS, Morris A, Nazemi MH, Pellett MA, du Plessis J, Quan YS, Raghavan SL, Roberts M, Romonchuk W, Roper CS, Schenk D, Simonsen L, Simpson A, Traversa BD, Trottet L, Watkinson A, Wilkinson SC, Williams FM, Yamamoto A and Hadgraft JD. (2005) Inter- and intra-laboratory variation of in vitro diffusion cell measurements: An international multi-centre study using quasi-standardised methods and materials. Journal of Pharmaceutical Sciences 94: 632-638.
- Fitzpatrick, D Corish J and Hayes B (2004) Modelling skin permeability in risk assessment – the future Chemosphere 55: 309-1314.
- Jakasa Y Mohammedi N Kruse J and Kezic s (2004) Percutaneous absorption of neat and aqueous solutions of butoxyethanol in volunteers Int Arch Occup and Environ Hlth 77: 79-84.
- Jones A (2004) Final report CEFIC Workshop on methods to determine dermal penetration for human risk assessment www.iom.
- Lee FW, Earl L, Williams FM (2001) Interindividual variability in the percutaneous penetration of testosterone through human skin in vitro. Toxicology 168: 63.

- Traynor, MR, Ball, E, Wilkinson S and Williams FM (2005) Influence of dilution with water on the dermal absorption of glycol ethers in vitro, paper in preparation.
- 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 , Neilsen 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.
- Wilkinson, SC and Williams FM. Effects of experimental conditions on percutaneous absorption of glycol ethers. International Archives of Environmental & Occupational Health 2002, 75**:** 519-527.
- Wilkinson S.C, Maas, WJM, Nielson, J, Greaves, LC, van der Sandt JJM, Williams FM (2005) Interactions of skin thickness and physicochemical properties of test compounds in percutaneous penetration studies submitted Int Arch Occupational Env Hlth, accepted.
- Wilkinson, SC Wilkinson RE and Williams FM (2005) Percutaneous absorption of lipophilic compounds in aqueous vehicles – influence of dose volumes Abstract Occupational and Environmental Exposures of Skin to Chemicals 2005.
- Williams Faith M et al (2004) EDETOX Project Evaluation and predictions of dermal absorption of toxic chemicals final report www.ncl.ac.uk /edetox.
- Williams Faith M (2004) EDETOX Evaluation and Predictions of dermal absorption of toxic chemicals International Arch Occup Environ Hlth 77: 150-151.



THE ASSESSMENT OF THE DERMAL BIOAVAILABILITY OF CHEMICALS BY USING APPROPRIATE IN VITRO METHODS

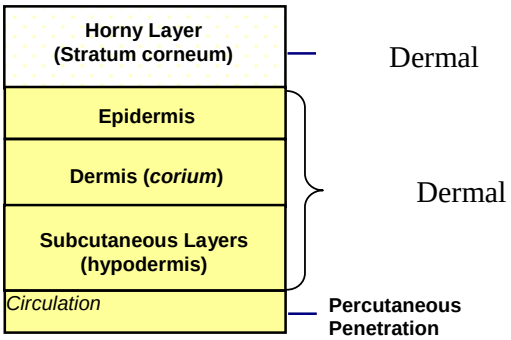
Wilfried Steiling
Henkel KGaA, Düsseldorf, Germany

Introduction

Following the increasing scientific interest in risk rather than hazard assessment, and convinced of the three R's approach for animal testing (Refine, Reduce, Replace), an *in vitro* method for the assessment of dermal absorption and percutaneous penetration of topically applied chemicals was developed. As a first step a comparison of methods currently used by the European cosmetic industry and described in the scientific literature has been performed. The experimental details and recommendations were published as a COLIPA Guideline in 1995[1,2]. By an international inter-laboratory comparison study[3], and with nearly a decade of experience within the cosmetic industry, the robustness and relevance of this method have been confirmed.

For the safety evaluation of chemicals, knowledge of their bioavailability is crucial, in addition to recognized intrinsic toxicological potentials. This systemic availability is represented by the quantity of topically applied chemicals and in particular of cosmetic ingredients found in the living epidermis and/or dermis and in the circulatory fluids. To discriminate the portion bound to the horny layer, the *stratum corneum* (s.c.), from that amounts found in deeper tissue layers, three terms have been defined (Fig. 1): the "dermal adsorption" (on or within the s.c.), the "dermal absorption" (within the living epidermis/ dermis) and "percutaneous penetration" (substance passing through the skin).

Figure 1. *Important terms*



Following the established routine testing procedures and the 3R principles, the assessment of dermal absorption / percutaneous penetration should be carried out *in vitro*. Both, the use of human skin from cadaver or cosmetic surgery[4] and the use of excised pig skin[5], the latter yielding comparable results and being of much easier access on a frequent basis, is recommended for such tests.

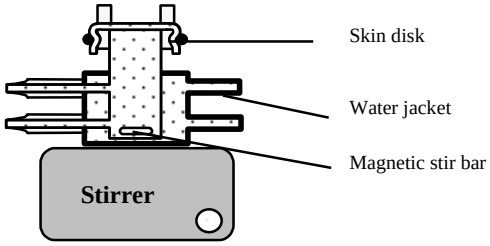
Methods

To mimic *in vivo* exposure conditions and to take into consideration the influence of specific chemicals on the dermal transfer, the test substance has to be assessed in an appropriate solvent and/or in a representative standard formulation. Additionally, the applied dose per skin area, as well as the duration and exposure condition (open or occlusive), should mirror as closely as possible the

intended use situation. All of these details have to be laid down in the test protocol to follow the principles of Good Laboratory Practice (GLP) and to find official acceptance of test results.

Skin disks, either human or porcine, are fixed in penetration cells, separating the donor from the receptor chamber (Fig. 2). Since these penetration processes through the skin are known to be passive and assuming the limited impact of skin-specific metabolism on normal skin penetration, properly frozen skin can be used strikethrough up to at least 3 months after excision. This option helps to standardize the skin disks (e.g. about 120 disks per pig).

Figure 2. *Scheme of a penetration cell*



The test chemical as such or within a standard formulation is topically applied to the horny layer, the upper outside of the skin. The receptor solution, ideally a sufficient solvent for the test chemical, in direct contact with the deeper skin layers, should be chemically inert and without compromising the skin integrity during the test run.

The exposure of the test chemical should be terminated by careful rinsing, or for cosmetic ingredients like hair dyes[6], by sensitive washings with a mild shampoo. Such discontinuation of the exposure has to be performed e.g. after 30 min. for rinse-off and after 24 hours for leave-on cosmetic raw materials to be close to the intended use (expected exposure) conditions.

The sampling, either continuously or at fixed time points during the study's run gives kinetic data to draw the penetration diagram[6], demonstrating the time related passing of the test chemical through the skin. The total test run should cover an appropriate time span (normally 24, but up to 48 hours) to be able to consider any retarded delivery from possible deposits in specific skin layers into the receptor fluid after termination of exposure.

At the end of the study, exposed skin samples are carefully rinsed e.g. with water, blotted with appropriate tissue papers and than tape-stripped to remove the horny layer with any adsorbed test substance. Normally, 10-15 tape strips are sufficient to remove an appropriate quantity of s.c. To obtain the amount defined as bioavailable, the residual skin is analyzed, often after separation of epidermis and dermis, for absorbed and the receptor fluid for penetrated test substance.

It should be stressed that appropriate analytical methods are essential to be able to measure the test chemical, especially in the complex biological matrix of the skin. When possible, the use of radio-labeled test substances should be preferred, to increase the effectivity of analytical detection and to reach a mass balance of at least 85%.

Discussion and recommendations

For risk and/or safety assessment of topically applied chemicals it is of main interest to know, in addition to their toxicological profile, their systemic availability after dermal contact. Specific

exposure data hereon is needed to mimic experimentally the intended use condition or the foreseeable exposure scenario, respectively[7].

The amount adsorbed to the *stratum corneum* is taken separately from the amounts found in epidermis and dermis and those having penetrated the skin and found in the receptor fluid. This latter amount of chemical, which would normally reach the circulatory fluids, is defined as systemically available. Due to the vascularisation of the dermis and its close association with the living epidermis, the quantity found in these skin layers is added to the proportion, which is regarded as bioavailable. It should be mentioned that the amounts found in skin appendages like sweat- or sebum glands or hair follicles and shafts are taken as systemically available as a conservative assumption. The exclusion of adsorbed test substance is justified by two aspects: firstly the *stratum corneum* consists only of death cells, corneocytes without any contact with the circulation in living skin, and secondly, the physiological process of desquamation, which leads to continuous renewal of this skin layer[8] under *in vivo* conditions.

It should be mentioned that any mechanical or physiological skin defect could affect the bioavailable of exposed chemicals. This would result in an increase of the margin of exposure within the risk and/or safety assessment.

The use of skin samples of different individual donors and replicate measurements are helpful to consider the biological variation in thickness and appendages, which is well known for human skin. Based on our experience 4 replicates of each of two donors should be appropriate.

If information exists on specific skin metabolism of the test substance, e.g. the cleavage of esters, the use of fresh skin may be preferable to frozen skin, to consider any potential impact of metabolic activity in skin on the absorption and penetration.

The described *in vitro* method cannot, of course, be employed for the assessment of systemic distribution and elimination of absorbed and/or penetrated test chemicals. To answer such questions, the additional use of a standardized *in vivo* model is recommended as an appropriate tool.

The use of artificial skin models instead of excised skin may in future be helpful to run these *in vitro* studies. But not all of these models are currently sufficiently standardized and quite often their barrier properties are not yet acceptable.

Conclusions

Today the use of excised human or pig skin is routinely recommended for cosmetic ingredients like UV-filters, preservatives and hair dyes by the *European Scientific Committee on Consumer Products* (SCCP)[9]. In 2001 this *in vitro* method was in principal accepted by the OECD and was finally published as the OECD guideline no. 428 in 2004.

References

1. Beck H, Benech-Kieffer F, Bracher M, Diembeck W, Dupuis J, Lovell W and Steiling W, Cosmetic Ingredients: Guidelines for Percutaneous Absorption / Penetration, COLIPA Guidelines, 1–36 (1995).
2. Diembeck W, Beck H, Benech-Kieffer F, Courtellemont P, Dupuis J, Lovell W, Paye M, Spengler J and Steiling W, Test Guidelines for the *in vitro* assessment of dermal absorption and percutaneous penetration of cosmetic ingredients. Food and Chemical Toxicology, 37, 191–205 (1999).
3. Beck H, Brain K, Dressler W, Grabarz R, Green D, Howes D, Kitchiner L, Pendlington R, Python M, Schröder K, Sharma R, Steiling W, Sugimoto K, Walters K and Watkinson A, An

Interlaboratory/Interspecies Comparison of the Percutaneous Penetration of [^{14}C]-p-Phenylenediamine (PPD) from a Hair Dye Formulation, *In Vitro*, In: Perspectives in Percutaneous Penetration, Vol. 7a, Edit. Brain K.R. and Walters K.A., STS Publishing, Cardiff, UK (2000).
4. Benech-Kieffer F, Wegrich P, Schwarzenbach R, Klecak G, Weber T, Leclaire J and Schaefer H, Percutaneous absorption of sunscreens *in vitro*: interspecies comparison, skin models and reproducibility aspects, Skin Pharmacology and Applied Skin Physiology 13, 324-335 (2000).
5. Steiling W, Kreutz J and Hofer H, Percutaneous penetration/dermal absorption of hair dyes *in vitro*, Toxicology In Vitro 15, 565-570 (2001).
6. Wolfram LJ and Maibach HI (1985) Percutaneous penetration of hair dyes. Archives of Dermatological Research 277, 235- 241.
7. Steiling W, The bioavailability of cationic azo hair dyes – application of the new in vitro method, Proceedings of the 4th World Congress on Alternatives and Animal Use in the Life Sciences, New Orleans/USA, August 2002, Alternatives to Laboratory Animals (ATLA) Supplement 1B, 32, 689-692 (2004).
8. Schaefer H and Redelmeier TE, Skin Barrier – Principles of Percutaneous Absorption. Karger-Verlag Basel, Freiburg, Paris, London, New York (1996).
9. SCCNFP Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers, Opinion Concerning: Basic Criteria for the in vitro assessment of dermal absorption of cosmetic ingredients (SCCNFP/0750/03 Final), Adopted by the SCCNFP during the plenary meeting of 22 October 2003 (2003).

QSAR, REACH AND THE PREDICTION OF SKIN PERMEABILITY

Mark TD Cronin
School of Pharmacy and Chemistry,
Liverpool John Moores University,
Liverpool, England

(Quantitative) structure-activity relationships ((Q)SAR) attempt to relate the biological activity of a chemical, or series of chemicals, to their physico-chemical and / or structural properties. QSARs are normally statistical algorithms that formalize these relationships allowing for some form of predictive model. They are based on techniques varying in complexity from regression analysis to neural networks. These techniques have been applied widely in product development to make more efficacious compounds, and for risk assessment to predict toxicity and fate. There are many advantages to the use of QSARs, including the facts that once created they are cheaper than traditional (*in* vivo) tests, and they negate the use of animals. However, their successful use is often a skilled process requiring an appreciation of the limitations of the model. For a full background to the science of QSAR, the reader is referred to Cronin and Livingstone (2004) as a starting point.

QSARs have been developed for numerous endpoints relating to risk assessment. These include both toxicity and fate effects. In terms of modeling the skin penetration of chemicals, QSARs are best able to model the intrinsic permeability of chemicals. This is because models are able to treat this as a steady-state phenomenon, which is analogous to physico-chemical effects such as passive diffusion. The skin penetration endpoint that is most frequently predicted is the skin permeability coefficient.

There have been many attempts to predict permeability coefficients. Good reviews of QSARs for skin penetration exist e.g. Cronin (2005), Geinoz et al. (2004) and Moss et al. (2002). In terms of the use of QSAR, preference is normally give to transparent and mechanism-based models, even if these are compromised in terms of statistical fit (Cronin and Schultz 2003). Approaches to predict skin permeability have ranged from the use of small local data sets e.g. for congeneric series to larger series of compounds. More applicable in terms of consumer products and forthcoming regulations, however, are the more generally applicable models. Flynn (1990), for instance, proposed a qualitative scheme to estimate permeability coefficient (K_p). Indeed, many of the more reliable QSAR studies have been based around data originally collated by Flynn (1990) and extensions of these data. The most relevant models are based on algorithms formulated as follows:

$\log K_p = a$ Hydrophobicity - b Molecular Size + constant

Where a and b are the regression coefficients.

Hydrophobicity is well characterized by the logarithm of the octanol-water partition coefficient ($\log K_{ow}$) and molecular size by parameters such as molecular weight (MW). This approach led Potts and Guy (1992) to develop the following model:

$\log K_p = 0.71 \log K_{ow} - 0.0061$ MW - 6.3
$n = 93$ $r^2 = 0.67$

where:

n is the number of observations
r^2 is the coefficient of determination

The model has been improved upon and refined by various workers, as reviewed by Cronin (2005). These latter approaches have improved the statistical fit of the models by the rationalizing of the data set (updating erroneous values) and by adding further parameters. The data set has been expanded in particular by efforts such as the EDETOX project (Fitzpatrick et al., 2004). A computerized version of the modified Potts and Guy equation is also available in the DERMWIN software, which is part of the EPISUITE software from the United States Environmental Protection Agency (US EPA), and can be downloaded free of charge from http://www.epa.gov/oppt/exposure/docs/episuitedl.htm.

QSARs for toxicity and fate are applied widely by regulatory agencies worldwide (Cronin et al, 2003a, b). Within Europe greater application is anticipated as a result of the forthcoming Registration, Evaluation and Authorisation of Chemicals (REACH) legislation. It is foreseen that QSARs could reduce greatly the cost of, and number of animals used in, REACH. As a result there is an impetus to provide guidance for the use of predictions from QSARs for regulatory purposes. To this end, the Organization for Economic Co-operation and Development (OECD, 2004) has ratified a series of principles for the assessment of the validity of a QSAR. These state that a (Q)SAR should be associated with the following information:

1. a defined endpoint
2. an unambiguous algorithm
3. a defined applicability domain
4. appropriate measures of goodness-of-fit, robustness and predictivity
5. a mechanistic interpretation, if possible

As such, these criteria provide a framework with which to evaluate (Q)SARs. However, it should be noted that the endpoints required by REACH do not include skin permeability directly, and that this is more likely to be important for risk assessment and the determination of exposure. Further, there is considerable debate regarding the value of a prediction of permeability coefficient in itself (these latter issues are described elsewhere in this volume).

With regard to skin permeability, an evaluation of QSARs is on-going through 2005 as part of a contract funded by the European Chemicals Bureau. There are a number of important issues when evaluating a predictive model, high amongst these is the concept of the applicability domain i.e. the structural, physico-chemical and biological space defined by the training set. These concepts are well described by Netzeva et al (2005). Whilst the data sets used to develop QSARs for permeability coefficients are expanding, the applicability domain has yet to be sufficiently defined. It is probable that a range of values i.e. the maximum and minimum of each descriptor will be too simplistic, and a more non-linear description of the domain is required. Even within this description of the domain there may be areas where greater confidence can be assigned to modeling and thus the predictions. A full assessment of the applicability domain of these QSARs is lacking at this time. Specifically with regard to the use of QSARs in REACH, there is a need for guidance by the beginning of 2007. It is envisaged that this will be provided by the OECD in collaboration with the European Chemicals Bureau.

Conclusions

Structure-based prediction methods, known as QSARs are widely available for skin permeability coefficients. Models developed following paradigms such as that described by Potts and Guy (1992) are general in nature and robust. However, as with all predictive models, QSARs for skin permeability coefficients are very dependent on data quality and other issues e.g. vehicles, test protocol etc. There is a probability that QSARs will be applied widely in the forthcoming REACH legislation and there may be a role for predictions of skin penetration in exposure assessment (although it is not an endpoint specified in REACH). Further consideration will be required as to how to utilize permeability coefficients for risk assessment and how to assign confidence to a prediction.

References

- Cronin MTD (2005) The prediction of skin permeability using quantitative structure-activity relationships (QSARs). In: Riviere J (Ed) *Dermal Absorption Models in Toxicology and Pharmacology*, CRC Press, Boca Raton FL, USA, in press.
- Cronin MTD, Jaworska JS, Walker JD, Comber MHI, Watts CD, Worth AP (2003b) Use of quantitative structure-activity relationships in international decision-making frameworks to predict health effects of chemical substances. *Environmental Health Perspectives* 111: 1391-1401.
- Cronin MTD, Livingstone DJ (2004) *Predicting Chemical Toxicity and Fate*. CRC Press, Boca Raton, FL, USA.
- Cronin MTD, Schultz TW (2003) Pitfalls in QSAR. *Journal of Molecular Structure (Theochem)* 622: 39-51.
- Cronin MTD, Walker JD, Jaworska JS, Comber MHI, Watts CD, Worth AP (2003a) Use of quantitative structure-activity relationships in international decision-making frameworks to predict ecologic effects and environmental fate of chemical substances. *Environmental Health Perspectives* 111: 1376-1390.
- Fitzpatrick D, Corish J, Hayes B (2004) Modelling skin permeability for risk assessment – the future. *Chemosphere* 55: 1309-1314.
- Flynn GL (1990) Physicochemical determinants of skin absorption. In Gerrity TR and Henry CJ (Eds) *Principles of Route-to-Route Extrapolation for Risk Assessment*. Elsevier, New York. pp. 93-127.
- Geinoz S, Guy RH, Testa B, Carrupt P-A (2004) Quantitative structure-permeation relationships (QSPeRs) to predict skin permeation: a critical evaluation. *Pharmaceutical Research* 21: 83-92.
- Moss GP, Dearden JC, Patel H, Cronin MTD (2002) Quantitative structure-permeability relationships (QSPRs) for percutaneous absorption. *Toxicology in Vitro* 16: 299-317.
- Netzeva TI, Worth AP, Aldenberg T, Benigni R, Cronin MTD, Gramatica P, Jaworska JS, Klopman G, Marchant CA, Myatt G, Nikolova-Jeliazkova N, Patlewicz GY, Perkins R, Roberts DW, Schultz TW, Stanton DT, van de Sandt JJM, Tong W, Veith G, Yang C (2005) Current status of methods for defining the applicability domain of (quantitative) structure-activity relationships. The report and recommendations of ECVAM Workshop 52. *ATLA* 33: 152-173.
- OECD (2004) Report from the Expert Group on (Quantitative) Structure-Activity Relationships [(Q)SARs] on the principles for the validation of (Q)SARs. OECD Environment Health and Safety Publications, Series on Testing and Assessment, Paris, France, p. 206 (available from http://appli1.oecd.org/olis/2004doc.nsf/linkto/env-jm-mono(2004)24).
- Potts RO, Guy RH (1992) Predicting skin permeability. *Pharmaceutical Research* 9: 663-669.

MODELLING SKIN PENETRATION: QSARS AND MATHEMATICAL MODELS

John Corish
Department of Chemistry, Trinity College
University of Dublin, Dublin2, IRELAND

Abstract
The results relating to the utilization of experimental skin penetration data measured in the EDETOX [1] project are reviewed. On their own these data were found not to be adequate to define a new QSAR to predict skin permeability but when they were added, in conjunction with other improved data, to an existing large database they confirmed the value of a QSAR in the well-established Potts and Guy format to predict K_p values [2]. In addition, the use of two mechanistic mathematical models to analyze and interpret new permeation data for a number of compounds is reported. They are used to extract values for the stratum corneum/vehicle partition coefficient and the diffusivity of the penetrants using both infinite and finite dose experimental data. The results calculated by the two models are in good agreement and should provide more reliable parameters for the skin penetration process. One of the models is also capable of using such parameters to produce a wide range of information for realistic occupational and leisure exposure regimes.

The EDETOX project and QSARS

New dermal penetration data for twenty one compounds were measured in the EDETOX project. Initially the values of the molecular weights (MW) of the compounds, their K_{ow} values and the measured K_p values were used in an attempt to establish a QSAR of the general Potts and Guy form:

$$\log K_p = a + b \log K_{ow} + c\, MW$$

which could be used to predict the K_p of compounds for which no penetration data had been measured. The data were examined using linear regression analysis with the three data fields ($\log K_p$, $\log K_{ow}$ and MW for each compound). However none of the coefficients apart from the intercept had acceptable significance in each others presence (t-values), and the p-value related to the F-statistic indicates that there is little evidence for a linear relationship between the permeability and the other variables. It is apparent that that this dataset is not of sufficient size to be capable, on its own, of defining a useful QSAR in the context of the current state of the art in the field. However these new data for 21 compounds taken from the EDETOX project were then combined with a set of data for 162 compounds taken from references [3] to [6]. A new data set for 181 compounds was formed – where there were duplicates the EDETOX data were chosen. These data were found to define an acceptable QSAR of the Potts and Guy form with the following parameters:

$$\log K_p \,(\text{cm hr}^{-1}) = -2.3160 + 0.7415 \log K_{ow} - 0.0098\, MW$$

The scatter plots indicated possible linear relationships with the expected slopes and the linear least squares fitting was almost as good as had been achieved with the original smaller datasets. The EDETOX points were not more widely scattered nor did they appear to fit any better than the others and no strong conclusions can be drawn from quantile/quantile plots or from other statistical diagnostics. The successful incorporation of these twenty-one new independent and carefully measured data into the previously existing QSAR of the Potts and Guy form serve to validate it as the

best available general purpose linear QSAR and to emphasize its robustness. It should increase the level of confidence that can be placed in the ability of this QSAR to predict the values of K_p for compounds on the basis of their molecular weight and octanol-water partition co-efficient.

Mechanistic models with infinite and finite permeation data

Two newly developed mechanistic models have been used to analyze and interpret both infinite and finite dose permeation data measured especially for this purpose. They are both one-dimensional diffusion models with four compartments: vehicle, stratum corneum, viable epidermis and receptor. The models were implemented in tandem for the purpose of comparison and validation. The two layers of the skin (VE and SC) are each characterized by a thickness and a diffusion coefficient. The values of the diffusion coefficients depend on the size of the penetrant. The distribution between the different compartments is determined by partition coefficients, which depend on the lipophilicity of the penetrant and on the composition of the different compartments. Both models were adapted from their original versions so that they ran with an almost common set of parameters and their results could be reliably compared. The first model, which we shall call the Krüse model, was first developed by Krüse and Verberk [7] and run with the **ACSL** software package: here it was implemented using the Berkeley Madonna package. The second model, which we shall call the AR model, was based on two papers by Anissimov and Roberts [8, 9]. In this implementation its interface was altered to give parameters analogous to those used in the Krüse model and it was run on two platforms, both different from the Micro Maths SCIENTIST used by the original authors. In the first of these a numerical Laplace inversion routine was obtained from Mathematica. Symbolic solutions to the model equations were obtained in Laplace space and numerically inverted. In the second, the model was implemented in Standard C++ on the Linux platform, using a numerical Laplace inversion routine written in-house. C++ routines for cumulative absorption, flux, and other variables were then attached to the R statistical environment via its C API.

Experimental data, measured in both infinite and finite dose experiments, for testosterone, parathion, malathion, caffeine and triclosan were extensively analyzed using these models [10] to give values for the diffusivity through the stratum corneum, D_{sc}, and the partition coefficient between the vehicle and the stratum corneum, $K_{sc/w}$. Both the cumulative quantity absorbed and flux curves were analyzed with the results from the two models being in very good agreement. Fitting the infinite dose experimental data was typically found to give two solutions in which the values of D_{sc} were essentially the same but with very widely different values for $K_{sc/w}$. Fitting the finite dose data was less satisfactory but these data could be used to distinguish between the two solutions from the infinite dose data using the following method. When the parameters from the two solutions to fitting the infinite dose data were used to calculate the finite dose curves, by appropriately altering the concentration in the vehicle, one set was found to reproduce the data well over approximately the first four hours of exposure while the other set failed completely to reproduce the experimental curve. The former set was then taken to be the parameters best representing the absorption process. It was also noted that the value for $K_{sc/w}$ chosen in this way could also be determined by the equation

$$K_{sc/w} = \min_i \left| K^i{}_{sc/w} - K_{QSAR} \right|,\ i \in \{1,2,\cdots\}$$

which compares the fitted value of $K_{sc/w}$ with the value expected according to Potts and Guy and Bunge and Cleek: $K_{QSAR} = K_{ow}{}^{0.74}$. This equation held true for each of the substances for which data was measured and fitted in this work.

The importance of the results summarized here and presented fully elsewhere [10] is that the parameters determined by the fitting can reproduce both the infinite and finite dose data and so are of

particular relevance in calculations of the delivery rates in occupational scenarios. The use of the mechanistic models also makes it possible to determine more accurate values for K_p by dividing the maximum flux calculated in the model by the corresponding vehicle concentration. The values so obtained have been shown to be different, in some cases by as much as an order of magnitude, from those resulting from a simple analysis in which the steady state flux, if such could be measured, was divided by the initial concentration in the vehicle. Such more accurate values for $K_{p,}$ and also more accurate and appropriate values for K_{ow}, should serve as the basis for the development of more accurate and dependable QSARs for skin penetration. Finally it should be noted that, in particular, the model developed by Krüse is quite flexible and can be used to simulate a variety of exposure scenarios, including those typical of occupational and leisure regimes. This means that the parameters governing the rate of skin penetration determined in the comprehensive fitting, and capable of reproducing penetration from both infinite and finite doses, can be used to make the predictions necessary for risk assessments from such dermal exposures. These predictions could include the quantity absorbed and passed into the receptor, the content of the skin reservoir, the effects of multiple exposures and the rates at which all of these processes occur.

References

[1] The EDETOX Project (Contract QLRT-2000-00196) of the Fifth Framework Programme of the European Commission.

[2] J. Corish, D. Fitzpatrick and D. Golden (in course of publication).

[3] Quantitative structure-activity relationships (QSARs) for the prediction of skin permeation of exogenous chemicals, Hiren Patel, Wil ten Berge and Mark T.D. Cronin, *Chemosphere*, 48 (5), 2002, 603-613.

[4] Quantitative structure-permeability relationships for percutaneous absorption: re-analysis of steroid data, Gary P. Moss and Mark T.D. Cronin, *Int. J. Pharm*, 238, 2002, 105-109.

[5] Skin permeability data: anomalous results, I.T. Degim, W. J. Pugh and J. Hadgraft, *Int. J. Pharm.*, 170, 1998, 129-133.

[6] Permeation of steroids through human skin, M.E. Johnson, D. Blankschtein and R. Langer, *J. Pharm. Sci.*, 84, 1995, 1144-1146.

[7] A theoretical exercise on the effects of formulation and application on the dermal uptake of agrochemicals, J. Krüse and C.W.E.Verberk in *Proceedings of Symposium Pesticide Formulation and Application – How to reduce side effects by means of New Technology*, 15/16 February 2001, The Hague, The Netherlands, (in Press).

[8] Diffusion modelling of percutaneous absorption kinetics. 1. Effects of flow rate, receptor sampling rate, and viable epidermal resistance for a constant donor concentration, Y. G. Anissimov and M. S. Roberts, J. Pharm. Sci., 88, 1999, 1201-1209.

[9] Diffusion modelling of percutaneous absorption kinetics: 2. Finite vehicle volume and solvent deposited solids, Y. G. Anissimov and M. S. Roberts, J. Pharm. Sci. 90, 2001, 504-520.

[10] Analysis, interpretation and extrapolation of dermal permeation data using mechanistically based mathematical models, Jacob Krüse, Darach Golden, Simon Wilkinson, Sanja Kezic and John Corish (in course of publication).

DERMAL EXPOSURE ESTIMATES: POSSIBLE IMPROVEMENTS

Wil ten Berge
Santoxar, Westervoort, The Netherlands

Introduction

This presentation deals with already existing ideas how to estimate the human exposure via the dermal route. The following points are addressed:

- Relevant items of exposure scenarios for workers and consumers
- Mechanism of dermal absorption and feasibility for QSAR
- Finite and infinite dose
- Skin absorption of mixtures

Dermal exposure scenarios

For quantitative risk assessment of exposure via the dermal route, the following elements are needed:

- Skin load per cm2
- Skin area
- Type of contact (vapor, liquid, solid)
- Duration of dermal contact
- Duration of exposure (not equal to duration of contact due to storage in the stratum corneum)

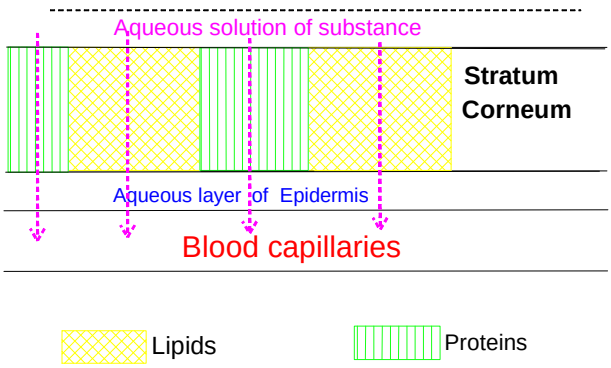
Mechanism of dermal absorption and feasibility for QSAR

In the European Technical Guidance Documents for Risk Assessment of Substances it is recommended to use 100% dermal absorption for the substances with a molecular weight of less than 500. No guidance is provided on the dermal absorption rate. This is surprising, because the dermal absorption rate is equivalent with the dose rate. Dose rate dependent toxicity is not an uncommon phenomenon in toxicology and should be considered in proper risk assessment.

The skin permeation coefficient from aqueous solutions (Kp in cm/hour) might be useful for estimating the maximum skin absorption rate. Multiplication of the Kp with the solubility in water (mg/cm3) results into an estimate of the maximum dermal flux (mg/cm^2/hour). Multiplication with the exposed skin area (cm2) provides the maximum skin absorption rate in mg/hour, which is equal to the dose rate via the dermal route.

Unfortunately, the aqueous skin permeation coefficient is reported only for a limited number of chemicals (ca. 150). In the scope of the future European REACH legislation risk assessment of all chemicals has to be done, including exposure via the dermal route. It is not well feasible to determine the skin permeation rate of about 30000 chemicals in the scope of REACH. So a QSAR (Quantitative Structure Activity Relationship) might be helpful in estimating the aqueous skin permeation coefficient.

In order to be able to derive a QSAR it is useful to consider the dermal permeation process in more detail. An outline can be found in the picture below, which is a rough sketch of the so-called Robinson model.



The substance may pass the protein and lipid part of the stratum corneum simultaneously. The preference for the one or the other is dependent on the lipophilicity. Lipophilic compounds will pass predominantly via the lipid part and hydrophilic compounds via the protein part of the stratum corneum, but both pathways are parallel. Finally the substance has to pass the aqueous epidermis, before being absorbed in the blood capillaries. The main resistance for permeation is in the stratum corneum. The permeation coefficient increases with lipophilicity. However, the water solubility is decreasing and this hampers the permeation of the epidermis.

In order to estimate the overall permeation coefficient it is assumed, that each part of the stratum corneum has its own permeation coefficient and also the aqueous epidermis. So the combination of these 3 permeation coefficients should finally result into the overall permeation coefficient. This is presented in the equations below.

$$Kp_{sk-water} = \frac{1}{\frac{1}{Klip + Kpol} + \frac{1}{Kaq}} \quad cm/hour$$

$$^{10}\log(Klip) = b1 + b2 * ^{10}\log(Kow) + b3 * Mw^{0.7}$$

$$Kpol = \frac{b4}{Mw^{0.7}} \qquad Kaq = \frac{b5}{Mw^{0.7}}$$

$Klip$ = *permeation coefficient lipid layer*

$Kpol$ = *permeation coefficients protein layer*

Kaq = *permeation coeffcient aqueous layer*

Kow = *octanol/water partition coefficient*

Mw = *molecular weight*

$b1, b2, b3, b4, b5$ = *regression coefficients*

This model may gain more confidence, if it is possible to derive the regression coefficients from experimental data. Experimentally observed permeation coefficients, published by Wilschut et al.

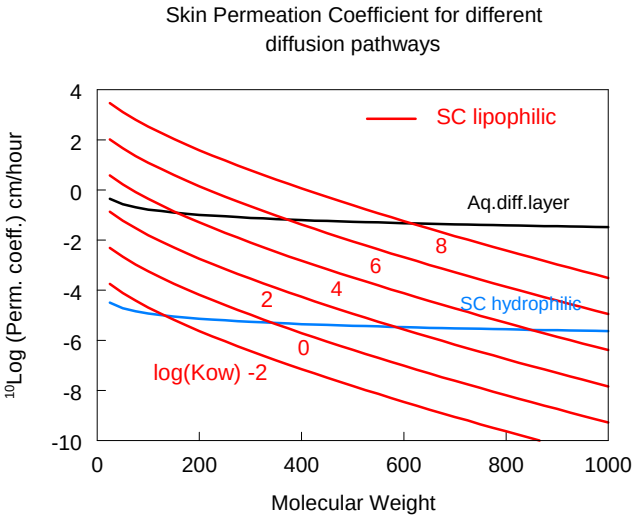
(1995) were used. Octanol/water partition coefficients were derived from EPIsuite (EPA, 2003). Non-linear regression produced the following results:

Filename of data = Wilschut et al (1995)
residual variance = 4.946E-01
degrees of freedom = 107
regression explained = 0.640

b 1 = -1.736E+00 Student t for b 1 = -8.982E+00
b 2 = 7.219E-01 Student t for b 2 = 8.234E+00
b 3 = -5.993E-02 Student t for b 3 = -8.751E+00
b 4 = 2.976E-04 Student t for b 4 = 1.051E+00
b 5 = 4.209E+00 Student t for b 5 = 5.257E-01

The regression coefficient b1, b2 and b3 apply to the permeation coefficient for the lipophilic part of the stratum corneum. The resulting equation is very similar to the Potts and Guy equation (1992). The regression coefficients b4 and b5 apply to respectively the Kp of the protein part of the stratum corneum and that of the aqueous epidermis.

It is interesting to study, in which domain of molecular weight and octanol/water partition coefficient the three permeation coefficients control the skin permeation rate. This is shown in the picture below.



The red lines represent the lipophilic permeation coefficient, dependent on molecular weight and the log(Kow), that is the log(octanol/water partition coefficient). This lipophilic permeation coefficient is the rate limiting step for compounds with a molecular weight of 200 and a log(Kow) between -1 and 4. This is more or less the domain of substances considered by Potts and Guy (1991). From the above graph it can be read for each substance, which of the three permeation coefficients are limiting the absorption rate dependent on the log(Kow) and the molecular weight. For very lipophilic compounds the rate limiting step will be the aqueous part of the epidermis. The transfer of a substance via the aqueous epidermis is heavily controlled by the solubility in the aqueous epidermis.

Finite and infinite skin dose and the use of Kp

How can the Kp of aqueous solutions play a role in risk assessment. This will be explained on the basis of the fate of an example compound in contact with the skin and on the basis of some well-known and widely adopted mathematical formulation of the skin permeation diffusion process.

The following steps are important:

- Contact time with the skin. The time of contact of a solid is controlled by the time of actual use and washing frequency. The time of contact with a liquid is controlled by the time of actual use, the time needed for evaporation of the skin and/or the washing frequency. In proper risk assessment, the contact time is increased with the lag time in order to take into account the absorption from the remaining substance in the stratum corneum.
- The absorption rate will be assumed to be the maximum absorption rate from a saturated aqueous solution of the substance (infinite dose).
- The substance might accumulate in the stratum corneum. In order to take this accumulated amount into account for absorption, the minimum duration of absorption is assumed to be twice the lag time, even if the contact time is less than 2 times the lag time.
- A check is made, that the estimated absorbed amount of substance is not more than the total skin load. If this condition is fulfilled the skin dose might be considered as an infinite dose. If the estimated absorbed amount via the skin is higher than the total skin load, it might be assumed that the skin dose has been a finite dose.

The equations below provide the tools for estimation of the absorbed amount of a substance by skin absorption.

$$Permeation\ coefficient\ \ Kp\ =\ \frac{Psc * D}{\delta}\quad (cm/hour)$$

$$Psc = 0.64 + 0.25 * Kow^{0.8}\quad (partition\ coefficient\ SC/water)$$

$$D\ =\ effective\ diffusion\ coefficient\ in\ stratum\ corneum$$

$$\delta\ =\ thickness\ stratum\ corneum\ \ (0.0015\ cm)$$

$$Lagtime\ \ in finite\ dose\ =\ \frac{\delta^2}{6D}\qquad (hours)$$

$$Time\ max.\ absorption\ \ rate\ \ finite\ dose\ =\ \frac{\delta^2}{6D}\quad (hours)$$

It should be kept in mind, that the Kp is the product of the partition coefficient of the substance between stratum corneum and water and the effective stratum corneum diffusion coefficient, divided by the thickness of the stratum corneum. The partition coefficient of the substance between stratum corneum and water (Psc) can be derived from a QSAR, controlled by the octanol/water partition coefficient, derived by (McKone and Howd 1992).

If the value of *Kp*, the δ and the *Psc* have been quantified, the effective diffusion coefficient can be estimated. The lag time is estimated from the relation $\delta^2/(6D)$.

In case of a finite dose it has been derived mathematically that the maximum absorption rate occurs at the lag time of an infinite dose. In practice this means, that the absorption of a finite dose occurs in a period of twice the lag time.

Skin absorption of mixtures

Water solubility is an important parameter for permeation of the epidermis. Especially for lipophilic compounds it might be quite rate limiting. Constituents of a mixture, which are also permeating the stratum corneum and enhance the solubility of the substance in the aqueous phase of the epidermis, will increase the absorption rate considerably. So if a substance is part of a mixture of chemicals in contact with the skin, it is worthwhile to consider:

- The permeation rate of each constituent next to the substance of interest. If not any constituent of a mixture except the substance of interest permeates the skin, the permeation rate is more or less similar to the permeation rate from an aqueous solution.
- The composition of the permeating mixture at epidermis level (what is the level of the constituents of the mixture in the epidermis in comparison with the composition of the applied mixture upon the skin).
- The solubility of the substance in the permeating mixture at the epidermis level. For instance, the permeation of a lipophilic substance from an aqueous solution in ethanol will be faster than from a pure solution in water, because also ethanol permeates the skin fast.
- An estimate of the permeation rate of the substance in the mixture. The corrected permeation rate might be the product of the aqueous skin permeation coefficient and the enhanced solubility due to permeation of constituents of the mixture in the epidermis.

Conclusions on skin permeation modeling

1. Published permeation coefficients of organic substances from aqueous solutions through human skin in vitro appeared to support a theoretical model for simulation of permeation of organic substances through the skin.
2. Modelling of skin permeation requires not only substance properties like the octanol/water partition coefficient and the molecular weight or the molar volume, but should also include diffusion kinetics.
3. Diffusion kinetics may provide additional understanding for the rate of permeation of gases, of liquids and of solid substances dissolved in water.
4. The model applies to non-ionized substances, which do not irritate, do not remove lipids from the skin and permeate faster than the substance is metabolized in the epidermis.
5. Model predictions are accurate within one order of magnitude. This is accurate enough to get some feeling for the contribution of dermal absorption in comparison with absorption by inhalation or ingestion.

A simple example of dimethyl nitrosamine

Dimethyl nitrosamine is a toxic volatile compound. Because of its carcinogenicity no experimental studies are likely to be performed. Still it is possible to make an assessment of the permeability on the basis of the model above. On the basis of the model above it has been estimated, that nitrosamine might have a maximum flux through the skin of 0.4 mg/cm2/hour. In addition, vapor is as fast absorbed via the skin as absorbed by inhalation in case of airborne exposure. This means that respiratory protection will decrease exposure to dimethyl nitrosamine for no more than 50% and is in fact not very efficient.

References

- McKone TE and Howd RA, 1992. Estimating dermal uptake of non-ionic organic chemicals from water and soil: I. Unified fugacity based models for risk assessment. Risk Analysis 12(4), 543-557.
- Potts RO and Guy RH, 1992. Predicting skin permeability. Pharmaceutical Research 9, 663-669.
- Wilschut A, Berge WF ten, Robinson PJ and McKone ThE, 1995. Estimating skin permeation. The validation of five mathematical skin permeation models. Chemosphere 30(7), 1275-1296.

EFFECT OF SKIN MODEL FORM ON PBPK PERFORMANCE

John C Kissel
AM Norman, JH Shirai, JA Smith
DEOHS, University of Washington, Seattle, WA, USA
Annette L Bunge
Chemical Engineering Department
Colorado School of Mines, Golden, CO, USA

Abstract
Only a few experimental investigations of dermal availability have been conducted *in vivo* in human volunteers. Those that have primarily involve dermal absorption of volatile organic compounds (VOCs) from aqueous solution. Compounds for which human *in vivo* experiments have been reported include chloroform (Gordon *et al.*, 1998; Xu and Weisel, 2004), methyl chloroform (Poet *et al.*, 2000a), trichloroethylene (Poet *et al.*, 2000b), toluene (Thrall *et al.*, 2002), methyl-tertbutyl ether (Gordon *et al.*, 2002), and haloketones (Xu and Weisel, 2004). Concentration of VOC in exhaled breath is the primary measure of absorption. Dermal permeation must then be estimated from observed breath measurements using some type of model. Investigators have typically adapted previously described multi-compartment physiologically based pharmacokinetic (PBPK) models to their cases by simply adding a skin compartment. Dermal permeability coefficients obtained by back-fitting PBPK models to breath concentration data in these experiments diverge to varying degrees from values obtained using the modified Potts-Guy relationship recommended by current U.S. Environmental Protection Agency (EPA) guidance. The modified Potts-Guy equation is a regression of permeability coefficients observed in *in vitro* experiments on molecular weight and octanol-water partition coefficient. Lack of correspondence between permeability coefficients determined by *in vivo* and *in vitro* methods is potentially problematic if regulatory protocols rely upon *in vitro* results. However, little attention has been given to date to the effect of alternative skin models on performance of the resulting PBPK models. Three versions of a skin model are considered here: the traditional approach, which treats skin as a continuously stirred tank reactor (CSTR) in which the VOC concentration is uniform throughout, a finite-difference (FD) model which treats the skin as a membrane with an internal concentration gradient, and an approximate membrane model which matches some performance characteristics of the true membrane model but is more easily implemented in the context of a PBPK framework. For illustration, these models have been applied to simulation of a scenario involving exposure to aqueous chloroform ($CHCl_3$). Observed differences in initial and post-exposure response time, storage and flux and effect on the apparent permeability coefficient are relevant to interpretation of output from PBPK models that include a skin compartment.

Methods
A multi-compartment physiologically based pharmacokinetic (PBPK) model was constructed. Three alternative representations of skin were utilized. The first is the traditional approach in which the skin is modeled as a continuously stirred tank reactor (CSTR). In a CSTR model, the blood exiting the skin is assumed to be in equilibrium with a single homogeneous skin compartment. The second model is an approximate membrane model constructed following the work of McCarley and Bunge (1998, 2001) and Reddy *et al* (1998). The version shown here is the "simplified time lag" (STL) model. The approximate membrane model is achieved by manipulation of coefficients of the CSTR model to give results that match a true membrane model for selected conditions. The last model is a

Finite Difference (FD) Membrane Model created by appending finite difference representations of exposed and unexposed skin to the PBPK model. All simulations shown here are based on the same scenario: 30 minutes of exposure to 40˚C water initially containing 91 ppb of chloroform; body weight of 72.6 kg; exposed skin surface of 21,300 cm^2; and permeability coefficient estimated by the modified Potts-Guy regression. Simulations were continued for an additional hour assuming removal of the external chloroform exposure source. All results were generated in MATLAB 7.0.1 (The MathWorks, Inc., Natick, MA).

Results

Figure 1 displays predicted time courses of chloroform concentration [ppb] in breath. In general, the CSTR model predicts more rapid rise in breath concentration upon initiation of exposure and more rapid decline upon cessation. Unlike the FD model, the CSTR model displays no delay in breath response.

Figures 2a-c display mass balances on the skin for the three approaches. Plots show predicted masses absorbed from water, stored in skin, and released to the blood stream. Skin storage is negligible in the CSTR model in comparison to the approximate or finite difference membrane models.

Figures 3a-c display disposition of mass absorbed into the blood stream. Absorbed mass must be metabolized, exhaled or stored in tissues other than the exposed skin. More rapid penetration of skin in the CSTR model leads to higher levels in internal tissues which in turn leads to greater mass exhaled and metabolized in the first 30 minutes.

Discussion

Prediction of highly variant breath profiles by CSTR and membrane models using the same value of the permeability coefficient is evident in Figure 1. It follows that back-fitting of a given data set to experimental data will yield variant estimates of the permeability coefficient. Estimates derived from *in vivo* experiments should therefore be interpreted accordingly.

Absence of holdup in skin accounts for prediction of more rapid exhalation of chloroform shown in Figure 1. The absence of skin storage in the CSTR model also explains the rapid post exposure decline in breath concentration.

Acknowledgements

This work was supported by US EPA via co-operative agreement R-82963201. Material presented here has not been reviewed by EPA and no Agency endorsement should be inferred. AMN and JAS have also received support via CDC/NIOSH Training Grant T42/CCT010418-11.

Selected References

Corey RA, Gordon SM, Wallace LA (2000). "Physiologically based pharmacokinetic modeling of the temperature-dependent dermal absorption of chloroform by humans following bath water exposures," *Toxicol Sci* 53:13-23.

Gordon SM, Wallace LA, Callahan PJ, Kenny DV, Brinkman MC (1998). "Effect of water temperature on dermal exposure to chloroform," *Environ Health Persp* 106(6):337-345.

Gordon SM, Brinkman MC, Satola JR, Wallace LA, Weisel CP, Clifford E, Shin JY (2002). "Controlled short-term dermal and inhalation exposure to MTBE and Dibromochloromethane," poster presentation, ISEA/ISEE annual conference, Vancouver, BC.

McCarley KD, Bunge AL (1998). "Physiologically relevant one-compartment pharmacokinetic models for skin. 1. Development of models," *J Pharmaceut Sci* 87(4):470-481.

McCarley KD, Bunge AL (2001). "Pharmacokinetic models of dermal absorption," *J Pharmaceut Sci* 90(11):1699-1719.

Poet TS, Corley RA, Thrall KD, Edwards JA, Tanojo H, Weitz KK, Hui X, Maibach HI, Wester RC (2000a). "Utility of real time breath analysis and physiologically based pharmacokinetic modeling to determine the percutaneous absorption of methyl chloroform in rats and humans," *Toxicol Sci* 54:42-51.

Poet TS, Corley RA, Thrall KD, Edwards JA, Tanojo H, Weitz KK, Hui X, Maibach HI, Wester RC (2000b). "Assessment of the percutaneous absorption of trichloroethylene in rates and humans using MS/MS real-time breath analysis and physiologically based pharmacokinetic modeling," *Toxicol Sci* 56:61-72.

Potts RO and Guy RH (1992). "Predicting skin permeability," *Pharmaceut Res* 9(5):663-669.

Reddy MB, McCarley KD, Bunge AL (1998). "Physiologically relevant one-compartment pharmacokinetic models for skin. 2. Comparison of models when combined with a systemic pharmacokinetic model," *J Pharmaceut Sci* 87(4):482-490.

Thrall KD, Weitz KK, Woodstock AD (2002). "Use of real-time breath analysis and physiologically based pharmacokinetic modeling to evaluate dermal absorption of aqueous toluene in human volunteers," *Toxicol Sci* 68:280-287.

Xu, X and Weisel, CP (in press). "Dermal uptake of chloroform and haloketones during bathing," *J Expo Anal Environ Epidemiol*, Aug 2004 pre-publication.

U.S. Environmental Protection Agency (1992). Dermal Exposure Assessment: Principles and Applications, Interim Report.

U.S. Environmental Protection Agency (2004). Risk Assessment Guidance for Superfund (RAGS), Volume 1: Human Health Evaluation Manual. Part E, Supplemental Guidance for Dermal Risk Assessment, Final Report.

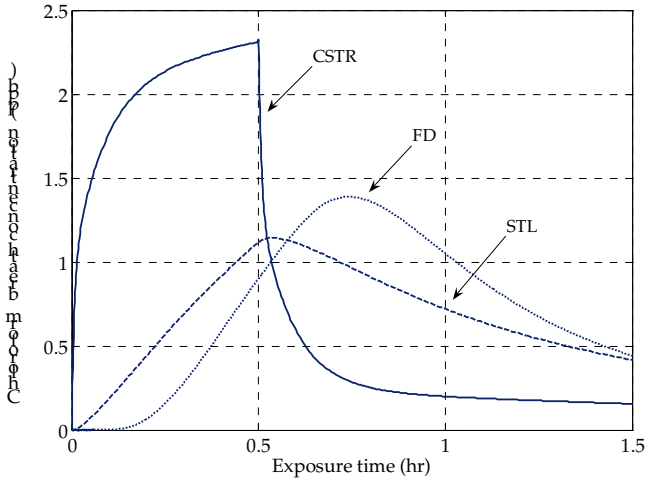
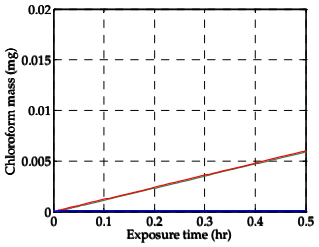
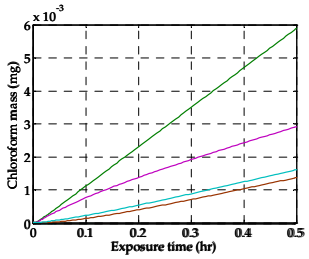


Figure 1. *Breath concentration profiles for the CSTR (solid line), approximate membrane (dashed line), and FD (dotted line) models.*



Figures 2a, 2b, and 2c. *Mass balance on skin for the CSTR, approximate membrane, and FD models. The green, red, and blue lines represent the net mass (mg) of $CHCl_3$ transferred from exposed stratum corneum to the bloodstream, mass (mg) of $CHCl_3$ transferred from water to stratum corneum, and mass (mg) of $CHCl_3$ stored in exposed stratum corneum, respectively.*



Figures 3a, 3b, and 3c. *Disposition of absorbed mass for the CSTR, approximate membrane, and FD models. The green, purple, brown, and aqua lines represent the net mass (mg) of $CHCl_3$ transferred from exposed stratum corneum to the bloodstream, mass (mg) of $CHCl_3$ stored in compartments other than exposed stratum corneum, mass (mg) of $CHCl_3$ metabolized, and mass (mg) of $CHCl_3$ exhaled, respectively.*

DERMAL EXPOSURE TO SOILS AND SOLVENT DEPOSITED SOLIDS: IS ABSORPTION PROPORTIONAL TO THE EXPOSED DOSE?

Annette L Bunge
Colorado School of Mines, Golden, CO, USA

1. Dermal absorption from contaminated soil

The results from a series of experiments of contaminated soils on silicone rubber membranes and human skin were described. These experiments indicate that dermal absorption only occurs from soil particles that have direct contact with the membrane or skin surface. Reporting results from contaminated soil experiments in terms of percent absorption can greatly underestimate the amount of absorption when the amount of soil on skin is less than required to completely cover it with soil. The study is described in a poster presented at the OEESC-2005 [1].

The potential health risk from dermal exposure to contaminated soils depends on the amount absorbed and the toxicological potency. Dermal absorption measurements from soils usually are reported as the percent of the amount of chemical applied (i.e., the *% absorption*), in which the amount of chemical is the product of the contaminant concentration on the soil (C_{soil}) and the amount of soil on the skin (M_{soil}/A). In actual exposures the amount of soil on skin is less than required to completely cover the exposed area. Despite this, absorption experiments are almost always conducted with enough soil to cover the skin surface with several layers. If chemical from the extra soil layers does not migrate to the skin, then the % absorption in an actual exposure could be much larger than observed in these multi-layer experiments. Thus, the goal of this study is to better understand the effect of M_{soil}/A on the % absorption.

The permeation of 4-cyanophenol (4CP, molecular weight = 119, octanol-water partition coefficient = 40) from soil was measured through human skin or silicone rubber membranes (SRM) used as a surrogate for skin. The SRM from Samco Silicone Products (Nuneaton, Warwickshire, UK) was about 360 μm in thickness. Split-thickness, human skin collected and frozen within 24 h of death was acquired from NDRI (Philadelphia, PA) and kept frozen at < -60°C until used. SRM or skin was clamped into Franz-type vertical diffusion cells (PermeGear, Bethlehem, PA) and equilibrated with the receptor solution for several hours. The diffusion area was 0.64 cm^2 and the average temperature of the cells was 32°C. The receptor solution, degassed deionized water, was introduced to the diffusion cell using a peristaltic pump (Ismatec, Cole Parmer Instrument Co., Chicago, IL). The solution from the receptor chamber was collected either continuously in 1-h intervals when the flow was constant (either 1 or 6 mL h^{-1} for applications of soil and aqueous solution, respectively), or periodically by pumping 30 mL h^{-1} for one minute every two hours (i.e., 0.5 mL). The latter sampling protocol was useful when the flux was small (as it is for soil on skin). The volume of the receptor chamber and outlet tube was determined to be < 0.2 mL.

The soil in this study was the < 250 μm fraction of a clay loam collected from Ft. Collins, CO, which contained approximately 1 weight percent organic carbon. This soil was contaminated with 4CP to a concentration (C_{soil}) of 300 mg g^{-1} by mixing 30 g of soil with 5 mL of 4CP in acetone. The acetone was then removed by evaporation. The amount of 4CP on the soil was then confirmed by extraction into acetonitrile and quantification of the extract by GC-FID.[1] Following a procedure described elsewhere, the solubility limit of 4CP in this soil, which had been aged for about 2 years, was

determined to be approximately 150 mg g^{-1}. Thus, the soil used in these experiments is supersaturated, meaning that an excess of 4CP is present.

Experiments were conducted with the whole contaminated soil (i.e., all particles < 250 μm) or with the 38 to 63 μm fraction of the whole contaminated soil. Soil was applied in an amount that produced multiple layers (40 mg cm^{-2}) or in smaller amounts between 0.2 and 8 mg cm^{-2}. In a few experiments, 40 mg cm^{-2} of soil was applied and then dumped off leaving a thin layer of particles that adhered to the skin or SRM. The mass loading of this *adhering layer* was approximately 1 to 2 mg cm^{-2}. Often, after a specified exposure time, the soil was removed, the membrane was rinsed with de-ionized water and then covered with a saturated aqueous solution of 4CP. After this, the experiment was continued for another 6 or 8 h. For each diffusion cell, the flux of chemical through the skin or SRM was monitored by HPLC analysis of the receptor solution collected over time.

Silicone Rubber Membrane (SRM). Early in the SRM experiments, the fluxes from the saturated solution and from the supersaturated soil are similar, as we might expect since the thermodynamic driving force for transfer through the SRM are the same. However, the flux from soil decreased in time, unlike the saturated solution, for which flux was constant. This decrease was even larger for the adhering layer.

After 12 h, about 67% of the 4CP from the adhering layer had penetrated the SRM. In the experiment with 40 mg cm^{-2} of applied soil, the flux decreased by 5 to 10-fold over the 12 h exposure even though only about 6% of the chemical had penetrated the SRM. This suggests that there was limited transfer of chemical from soil particles that do not have direct contact with SRM.

In a similar experiment, 40 mg cm^{-2} of soil was added to the adhering layer after 15 h and the diffusion cells that originally had 40 mg cm^{-2} of soil were shaken at 7, 10 and 15 h. The addition of soil to the adhering layer increased the flux almost to the value early in the experiment. A similar, but less dramatic response occurred when the multiple layers of the 40 mg cm^{-2} application was redistributed by shaking.

Chemical penetration of 4CP through the SRM increased with soil loading until the loading was ~8 mg cm^{-2}, after which further increases in soil loading had no effect. This is consistent with the idea that increasing the soil loading above the amount required to completely cover the surface does not increase dermal absorption. For the sieved soil, loading had no affect when it was larger than ~2 mg cm^{-2} (data not shown).

It was further shown that the cumulative mass of 4CP that penetrated through the SRM from the sieved soil (38-63 μm) and whole soil (< 250 μm) as a function of time and soil loading in experiments conducted on two different days. The results for experiments repeated on both days were similar, indicating reasonable day-to-day reproducibility. The penetration rate through SRM was greater from the sieved soil fraction than from the whole soil at a similar soil loading. Penetration through SRM from the sieved soil applied with a loading of 0.8 mg cm^{-2} was similar to that obtained for 3.1 mg cm^{-2} of the whole soil and was nearly twice as much as from 1.6 mg cm^{-2} of the whole soil. This suggests that the adhering layer consists mostly of small particles. Independent determination of 4CP concentration on the whole and sieved soil fraction showed no significant difference. This would indicate that increased 4CP penetration from the sieved soil was not due to an increased amount of 4CP on the smaller particles.

Skin. The contaminated soil was replaced after 12 h by saturated aqueous solution in an experiment similar to the one with SRM, but now withhuman skin. From saturated water, the flux after 8 h was approximately 50% larger through skin than through the SRM. However, much different than SRM, flux through skin from the contaminated soil was less than 10% of that from saturated water. Because absorption into skin from the contaminated soil was small, contaminant concentration on the soil changed only a little and consequently, the flux did not decrease in time as was observed in the SRM experiments. The reason for the much smaller flux from the contaminated soil compared to the saturated aqueous solution is unknown, although one possibility is that skin is heterogeneous while SRM is not.

Interestingly, the flux from saturated water reached steady state in 6 h in the SRM experiments, but continued to increase over 8 h in the skin experiments. By contrast, steady state flux through skin was easily established in 4 h in experiments in which saturated water was applied after a previous application of 38-63 μm sieved pure 4CP powder. It seems that the soil cleaning procedure leaves particles on the skin surface that affect absorption from water while they are slowly released from the skin surface.

Comparing the data, it is evident that the flux through skin from the contaminated soil is almost the same as from the pure powder, perhaps because this heavily contaminated soil is similar to pure powder. It is important to stress that flux through skin from the contaminated soil has a much greater variability than was observed in the SRM experiments. A 12-replicate experiment with 1.5 mg cm^{-2} of the sieved soil had a coefficient of variation of 50% in the cumulative amount of 4CP that penetrated the skin in 8 h. As a result, a plot prepared for skin showed no apparent correlation between flux and soil loading.

The conclusions were as follows:
Based on the observations described above, there appears to be little transfer of chemical from soil particles that do not have direct contact with the membrane or skin. Although SRM is commonly used as a surrogate for skin, absorption through skin shows some important differences compared to SRM. For SRM, the flux from contaminated soil is almost the same as from the saturated aqueous solution and pure powder, until absorption into the SRM reduces the concentration of chemical in the soil layer that is in contact with the SRM. Bringing fresh soil into contact the SRM surface increases the flux to its earlier value. For skin, the flux from contaminated soil is only about 10% of that from saturated solution, which is too small to cause the flux to decrease during the experimental times studied. For whole soil on the SRM studied, absorption increased to a maximum value at a soil loading of about 6 mg cm^{-2}, above which absorption did not increase further. A similar relationship between soil loading and absorption could not be established because of the large variability in the skin experiments.

2. Dermal absorption from solids deposited on the skin surface from solvents
Many experiments have been conducted in which a solid dissolved in a volatile solvent is placed onto skin. The solvent evaporates leaving behind the solid chemical on the skin surface. There is considerable experimental evidence that dermal absorption of solvent deposited solids is less than proportional to the applied dose. A description of the examples described is included in a poster presented at the OEESC-2005 [2], which are presented below along with the conclusions.

In many occupational exposures, a solution containing volatile components evaporates leaving a residue of nonvolatile components on the skin surface. Often these nonvolatile components are

solids. Furthermore, in many dermal absorption studies, solid compounds are applied to skin in a volatile solvent (e.g., acetone or ethanol), which subsequently evaporates leaving a film of the solid compound. It is almost always assumed that dermal absorption from solvent deposited solid films is proportional to the exposed dose (i.e., the amount of solid compound deposited onto the skin surface). Despite this, there is considerable experimental evidence that dermal absorption is proportional to concentration raised to a power less than one. In this poster, we review several dermal absorption studies of pesticides and drugs. Although the compounds and the formulations varied significantly, in all cases dermal absorption was proportional to the exposed dose raised to a power less than 1. Based on this result, we anticipate that reducing dermal exposure to solvent deposited solids may not produce proportionally less health risk. Here we examine the literature for answers to the question: Is dermal absorption from solvent deposited solid films, either the amount or rate, proportional to the applied dose? We present results from several studies involving a wide variety of chemicals that address this question.

Example 1 – Pesticide Registrant Studies. Pesticide registrants provide to the US Environmental Protection Agency (EPA) measurements of *in vivo* absorption of radiolabeled pesticides deposited onto the backs of male laboratory rats as prescribed in the *Zendzian* protocol (Zendzian, 2000). In these experiments, the concentrated formula and 2 or 3 dilutions (usually approximately 10-fold with water) are applied to skin as a small volume (not more than 10 mL cm^{-2}) on an area of at least 10 cm^2. Water evaporates soon after application. The exposed site is protected with a non-occlusive covering. During the exposure time, urine and feces are collected. At the end of the exposure time, the animal is sacrificed and the amounts are determined in the carcass, and on the skin and in the skin at the exposed site. Exposure times of 0.5, 1, 2, 4, 10 and 24 hours are typical. Four rats are usually measured for each exposure time and applied dose studied.
Typical of many pesticides that are solids at skin temperature, the percent that absorbed systemically

Table 1. *Properties of six conazoles*

	Pesticide	MW	T_{mp} (°C)	log$K_{o/w}$	S_w (mg/L)
C	Cyproconazole	292	107.5	2.91	140
D	Diniconazole	326	145	4.3	4.0
F	Fenbuconazole	337	125	3.23	0.2
H	Hexaconazole	314	111	3.9	17.0
P	Propiconazole	342	liquid	3.72	100
U	Uniconazole	292	155.5	3.67	8.41

decreased dramatically as the applied dose increased.

Dermal absorption of six different conazoles (fungicides) with the properties listed has been studied. Physical properties are listed in Table 1 and the amounts that penetrated skin (i.e., amounts systemically absorbed) in a 10 h exposure are shown in Figure 2 [2]. Dermal absorption of the 5 conazoles that are solids at skin temperature was less than proportional to the applied dose. That is, Dermal Absorption $\propto$ (Applied Dose)^b and $b < 1$.

In addition to this, we have statistically analyzed dermal absorption of these 5 conazoles along with 7 other pesticides (Table 2). All 12 are solids at skin temperature and were studied using the Zendzian protocol. These 12 pesticides did not damage skin, did not evaporate, and less than 50% dermally absorbed (i.e., the amount on the skin surface was always at least 50% of the applied dose). On average for these twelve pesticides, the amount absorbed was proportional to the square-root of the applied dose divided by the density of the pesticide. The amount absorbed was also proportional to the square-root of octanol solubility (as approximated by the product of the octanol-water partition coefficient and the water solubility).

Example* 2 – *Ibuprofen and Flurbiprofen. Akhter and Barry (1985) studied *in vitro* absorption of radiolabeled ibuprofen and flurbiprofen deposited onto human skin using acetone. Three or four different doses were applied in a small volume (< 22 mL/cm^2) of solution. Acetone evaporated soon (< 2 min) after application. Cumulative penetration into receptor solution (phosphate buffered saline at pH 7.4) was measured. They measured penetration through skin for 60 hours without occlusion and then for 40 more hours with occlusion (by parafilm). For Flurbiprofen the penetration rate was proportional to the applied dose raised to the 0.36 power. For Ibuprofen, penetration rate was proportional to the applied dose raised to the 0.71 power.

Table 2. *Properties of seven additional pesticides*

Pesticide	M	T_{mp} (°C)	log $K_{o/w}$	S_w (mg/L)	log($K_{o/w}S_w$)
Azionphos-methyl	317	140	2.96	8.4	3.88
Imazalil	297	53	3.82	180	6.07
Iprodione	330	134	3.00	13	4.11
Isoxaflutole	359	140	2.32	6.2	3.11
Lindane	291	113	3.72	7.3	4.58
Phosmet	317	73	2.95	25	4.35
Vinclozolin	286	108	3.00	2.6	3.41

Example* 3 – *Cortisone. Scheuplein and Ross (1974) studied the absorption of cortisone deposited from acetone onto human skin *in vivo*. They found that the *transfer coefficient*, defined as the % of the applied dose that penetrated skin per hr, decreased as the applied dose increased. The decrease was most dramatic for applied doses less than 30 mg cm^{-2}. Consistent with the previous examples, skin penetration was less than proportional to the applied dose. According to Scheuplein and Ross, "the transfer coefficient, is quite dependent on the quantity of material applied."

Example* 4 – *Various Drugs and Pesticides. Wester and Maibach (1999) reviewed the effect of applied dose on dermal absorption in a variety of systems (*in vivo*, *in vitro*, human, rhesus monkey and hairless dog). In the following studies of solvent deposited solids onto skin, the percent of the applied dose that dermally absorbed decreased as the applied dose increased.

- Testosterone, hydrocortisone and benzoic acid measured *in vivo* in rhesus monkeys and humans.
- Lindane measured in humans *in vivo*.
- N-diethyl-m-toluamide and sulfonamide were in hairless dog.
- Nitroglycerin from an unspecified skin source. In this study the % absorbed was approximately constant for applied doses < 1000 mg cm^{-2} but decreased for applied doses > 1000 mg cm^{-2}.

Dinoseb deposited onto rat skin *in vivo* was the single case for which the percent of the applied dose that absorbed did not decrease as the applied dose increased.

There is little experimental support for the assumption that the percent absorption is independent of applied dose. There is considerable experimental evidence that dermal absorption of solvent deposited solids is less than proportional to the applied dose: Experimental measurements of dermal absorption are often made on applied doses that are larger than real exposed doses. Extrapolating experimental measurements at these high doses to lower doses by assuming the percent absorption is constant can greatly underestimate dermal absorption (and the potential health risk).

3. The effect of vehicle

Dermal absorption should be proportional to the thermodynamic activity of the absorbing compound in the vehicle. An example was provided of saturated solutions of phenanthrene in two different vehicles: water and light viscosity silicone oil. Although the phenanthrene concentration in the silicone oil was nearly 10,000-fold larger than the concentration in water, dermal absorption was nearly the same, which is consistent with the fact that the thermodynamic activity of phenanthrene was almost the same in these two vehicles.

4. Dermal absorption estimates based on maximum flux provide a measure of the likelihood of dermal absorption that can be used as an alternative to either percent absorption or permeability coefficient values. The maximum flux approach uses estimated values of the permeability coefficient from water multiplied by the water solubility. As long as the vehicle does not alter skin too much, the maximum flux should represent the maximum absorption rate. If the concentration in a vehicle is less than saturated, then the flux would be proportional to the degree of saturation in the vehicle. Examples of the maximum flux approach were provided. These example calculations were compared with experimental data of dermal absorption of pesticides measured in rats *in vivo*.

References

1. Sandrine E. Deglin, Donald L. Macalady and Annette L. Bunge, Absorption from contaminated soil into skin and silicone rubber membranes, Programme book for OEESC-2005, Stockholm, Sweden.
2. Annette L. Bunge, Dermal exposure to solvent deposited solids: Is absorption proportional to the exposed dose?), Programme book for OEESC-2005, Stockholm, Sweden.
- Other references: see the posters in Programme book for OEESC-2005, Stockholm, Sweden.

COSMETICS: SKIN ABSORPTION DATA IN HUMAN RISK ASSESSMENT

Walter Diembeck
Beiersdorf AG, Hamburg, Germany

Definition: "Cosmetic Product"
Any substance or preparation intended to be placed in contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition. [93/35/EEC]

Definition "Cosmetic Ingredient"
Any chemical substance or preparation of synthetic or natural origin, used in the formulation of cosmetic products.
A cosmetic ingredient may be:
1- a chemically well-defined single substance with a molecular and structural formula
2- a complex preparation, requiring a clear definition and often corresponding to a mixture of substances of unknown or variable composition and biological nature,
3- a mixture of 1 and 2, used in the formulation of a finished cosmetic product. [93/35/EEC, SCCNFP/0321/00]

Data required from cosmetic ingredients suppliers
- chemicals: skin absorption data normally not required by EU dangerous substances legislation (Directive 67/458/EEC + amendments).
- cosmetics: skin absorption data required by cosmetic industry for safety dossier to be presented to SCC(NF)P (6th and 7th Amendment to Directive 76/768/EEC) esp. of "actives", e.g. colorants, preservatives, UV filters, substances with limitations (concentration, site of action).

Data required from the cosmetic industry
Skin absorption data must be provided by cosmetic industry in the safety dossier "TIF" (Technical Information File) which must be kept available by the manufacturer or importer of each cosmetic product within the EU and made accessible to the competent authorities of the Member States on demand (according to 6th and 7th Amendment to Council Directive 76/768/EEC) of all ingredients, in particular "actives", e.g. colorants, preservatives, UV filters, substances with limitations (concentration, site of action).

Safety assessment of a cosmetic product
Overall toxicologic profile of ingredients (remark: importance of purity, impurities, origin!), chemical composition and degree of exposure
for intended use and foreseeable misuse (e.g. shower gel used as shampoo, face care product used for hand or body care).

Exposure
Dermal exposure = process of contact between an agent and skin at an exposure surface over an exposure period.

Exposure assessment is a prerequisite:
topical application > dermal uptake (spray: inhalation!)
lipcare > dermal and additional oral uptake (ingestion)
oral care (e.g. mouthwash) > oral uptake.

Helpful questions:
- what (product)
- where (site of application)
- how (mode of application, e.g. spray, liquid, powder)
- how much (applied amount)
- how long (leave-on [hours, days], rinse-off [minutes])
- how often (daily ...)
- user (children <> grown-ups, consumer <> business)

Available human and animal tests
Studies can be performed in human volunteers in the case of low toxicity of cosmetic product / ingredient. Often, no human data are available.
For *in vivo* testing see:
- OECD TG 427 (2004)
- OECD Guidance Document for the Conduct of Skin Absorption Studies No. 28 (2004).

Available alternative methods
In vitro testing is preferably carried out on excised pig or human skin (avoid use of lab animals in cosmetic industry in EU due to politics).
For *in vitro* testing see:
- OECD TG 428 (2004)
- Diembeck at al., Fd.Chem.Tox. 37, 191-2005 (1999)
- OECD Guidance Document for the Conduct of Skin Absorption Studies No. 28 (2004)
- Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients [SCCNFP/0750/03].

***In vivo* and/or *in vitro* ?**
National regulatory authorities may have different preferences for *in vivo* and/or *in vitro* skin absorption studies.
Choice should be in line with requirements of authorities and depends on the situation to be evaluated (e.g. exposure different for leave-on vs. rinse-off products).
The test should mimick the intended use conditions.
European Union: According to the 7th Amendment to Dir. 76/768/EEC [2003/15/EC] the *in vivo* study will be prohibited for cosmetic ingredients from 11 March 2009 on (already now animal *in vivo* studies prohibited for finished cosmetic products).

In vitro method has been used in industry since many years for
- selection of suitable ingredients (BDF: also regarding skin irritation potential, phototoxicity)
- optimization of cosmetical formulations with respect to safety and efficacy (effect of vehicle!)
- in-house hazard identification and safety assessment
- prerequisite for compatibility testing on human volunteers
- regulatory purposes

Choice of vehicle depends on

- properties of ingredient of interest
  e.g. lipophilicity, solubility, stability
- intended use of product
  leave-on (e.g. sunscreen)
  rinse-off (e.g. hair-dyeing, cleansing)

Composition of vehicle influences

- delivery of ingredient(s) to skin
- extent of absorption / penetration
- acceptance of product by the consumer
- efficacy of product
- safety of product

Dose - activity analysis

(on a case-by-case basis, SCCNFP recommendation)

- amount / product / person / day
- amount / body weight
- amount / area
- Systemic Exposure Dosage [amount/kg body weight])
- Margin of Safety = NO(A)EL / SED

What we do in Beiersdorf

- *In vitro* penetration studies are integral part of cosmetic product development (also for dermatologics).
- Exposure to cosmetics is localized, quantifiable, voluntarily > realistic assumptions for known ingredients and products. Results expressed in [% applied dose] or [µg/...].
- BDF uses experimental data (mostly excised pig, sometimes human skin, no 3D-skin models) (mostly in-house) only
- No mathematical modelling in BDF because of complexity of cosmetic vehicles, lipophilic molecules, experienced shortcomings of mathematical models for our purposes at date.

Recommended literature

The SCCNFP's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation (5th Revision) [SCCNFP/0690/03 Final, adopt. 20 October 2003].

HOW DERMAL BIOAVAILABILITY DATA IS USED IN HUMAN RISK ASSESSMENT OF PESTICIDES/BIOCIDES

John Ross
Infoscientific
Carmichael, CA, USA

The large surface area and unique composition of the outer layers of the human body present both a significant barrier and some portals of entry to the interior (slide 3). Dermal absorption data has many sources. Early on (30+ years ago) methods were developed to study dermal absorption in humans (Feldmann and Maibach, 1974) and about this same time, it became evident that the dermal route contributed most exposure (Wolfe, 1976). As regulation of pesticide exposure evolved into quantifying absorbed dose (NAS, 1983), the regulatory community recognized the value of human and non-human primate dermal absorption data. Given a choice most knowledgeable regulators would prefer to have in vivo primate data, but infrequently request it and never require it. Less than 20% of all registered pesticides have any in vivo dermal absorption data, and <3% have any in vivo primate data. Further, primate data are frequently limited by having only a single dose tested while in vivo rat data usually has 3 dose levels spanning the expected range of exposure.

The lack of in vivo data and expense and ethical considerations in producing it has prompted the EU to adopt in vitro data in a tiered approach to dermal penetration in risk assessment. The first tier in such an approach is the default assumption of 100% bioavailability. If the 100% assumption produces unacceptable calculated risk, a second tier estimate using physicochemical parameters, e.g., molecular weight, log octanol/water partition coefficient or oral bioavailability is used. Those chemicals not passing Tier II screening can then be required to produce in vitro dermal absorption data, although in vivo data is given preference for Tier III assessments. Ultimately, if all else fails, biomonitoring can be used to most accurately measure absorbed dosage. The tiered approach just described for the EU is radically different in North America for Tier II and III. In North America, dermal toxicity data compared to a dermal dose is given preference over calculated absorbed dose that is compared to oral toxicity endpoints. Failing Tier II, the North American approach goes directly to in vivo dermal absorption as no in vitro data of any kind are allowed in regulation. As we can see from the varying regulatory approaches to the use of dermal penetration data, measures of dermal bioavailability can be assessed several ways depending on the need for accuracy (ranked in slide 7) in approximately the order of accuracy.

There are a number of methods to interpret in vivo dermal bioavailability data, and they are dependent on study design. Regardless of the method used, from a regulatory standpoint there is a desire never to underestimate resulting in "conservative" interpretation of the data. Three regulatory methods of data interpretation are discussed: the mass balance method (Zendzian, 1994), the plateau method (Thongsinthusak et al., 1999), and the Maibach method (Feldmann and Maibach, 1974). The plateau method and mass balance method tend to give the same numbers if excreta is collected until limit of detection is reached, and there is little dose remaining at the treatment site following wash off at termination. The Maibach method also is similar in result to the mass balance method (within ± 60% for six different compounds tested by both methods; Ross et al., 2005).

Clearly, the rat as a model for human dermal absorption is extremely conservative averaging five times the estimated bioavailability of humans using the same pesticides (Ross et al., 2001). The

variability introduced by using an inbred laboratory animal (e.g., the rat) under very rigid controlled conditions is typically less than that observed with the human population (see slide 12 for an actual comparison of results from both species for azinphos-methyl). However, the uncertainty introduced by using rat data as indicated above tends to be larger than the variability introduced from using human subjects. Ideally, to reduce uncertainty it would be desirable to have human dermal bioavailability data. However, for a variety of reasons, <3% of all registered pesticides/biocides have in vivo dermal data. An alternative now used in the EU as part of the tiered approach to refining dermal bioavailability estimates is in vitro absorption. Unfortunately, North American regulators will not currently use in vitro data and a concerted effort to demonstrate the utility of in vitro data using paired data sets of in vitro and in vivo rat and human data for the same compounds has not been undertaken. This is not because the data do not exist; rather it is because the data have never been adequately compiled for a reasonable number of compounds (n=6?).

Depending on the data available and the intended use, dermal bioavailability data is expressed several ways, although a very vociferous group opines that k_p is the "best" method. The use of dermal bioavailability data is varied depending on the desired application. For example, it may be used to estimate systemic dose from a dermal dose, or vice versa (estimating dermal exposure from an absorbed dose measured in a biomonitoring study). With the increased interest in children's exposure, dermal bioavailability must be distinguished from non-dietary oral exposure resulting from hand to mouth activities. Because the vast majority of systemic dosage occurs via the dermal route (high vapor pressure compounds excluded), it is feasible to use biomonitoring data generically, i.e., to incorporate it into dermal exposure databases e.g., POEM. To do this meaningfully, one must have a human dermal pharmacokinetic study, however.

The equation for calculating operator exposure when using a database e.g., POEM makes dermal bioavailability the singular variable that is chemical-specific, and therefore a very critical determinant of absorbed dosage. The methods for estimating post application or reentry systemic exposure whether for workers or residents are very similar. Increasingly, with standardization of transfer coefficients (e.g., US EPA's Policy 3.1), the dermal absorption factor is becoming more critical.

North American regulatory agencies have been using high dosage dermal toxicity studies as the basis for regulatory NOAELs for operators and residents. This regulatory approach is in contrast to the method used in the EU where an oral toxicity study NOAEL is compared to the absorbed dose calculated using a dermal bioavailability factor. These conflicting regulatory approaches can lead to differences in conclusions about pesticide/biocide safety, and must be resolved in the interest of science and harmonization. While the North American method nominally avoids route to route extrapolation, it ignores several factors that more than offset the uncertainty of route to route extrapolation. First, the gut excels at absorption due to peristalsis, dilution with digestive juices and a very large effective surface area. Secondly, absorption rate is limited when dermal dose exceeds a monolayer on the skin versus gut. Finally, irritation or tissue damage from high dermal doses and/or repeat applications confounds the interpretation of dermal dose toxicity studies.

References

- Feldmann, R.J., and Maibach, H.I. (1974). Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol 28: 126-32.
- NAS (National Academy of Sciences) National Research Council. (1983). Risk assessment in the federal
government: managing the process. National Academy Press, Washington DC.

- Ross, J.H., Driver, J.H., Cochran, R.C., Thongsinthusak, T. and Krieger, R.I. (2001). Could pesticide toxicology studies be more relevant to occupational risk assessment? Ann. Occup. Hygiene 45 (Suppl 1): 5-17.
- Ross, J.H., Driver, J.H., Harris, S.A., and Maibach, H.I. (2005). Dermal Absorption of 2,4-D: A Review of Species Differences. Reg. Toxicol. Pharmacol. 41: 82-91.
- Thongsinthusak T., Ross, J.H., Saiz, S., and Krieger, R. (1999). Estimation of Dermal Absorption Using the Exponential Saturation Model. Reg. Toxicol. Pharmacol. 29, 37-43.
- EPA (2000) Policy 3.10- Agricultural Transfer Coefficients, Revised August 7. Office of Pesticide Programs, Science Advisory Council for Exposure, Washington, DC.
- Wolfe, H.R. (1976). Field exposure to airborne pesticides. In *Air Pollution from Pesticides and Agricultural Processes* (R. E. Lee, Ed.), pp. 137-161. CRC Press, Boca Raton, FL.
- Zendzian, R.P. (1994). Dermal absorption of pesticides. Pesticide assessment guidelines, Subdivision F, hazard evaluation, human and domestic animals, Series 85-3. Health Effects Division, U.S. EPA, Washington, D.C., USA.

CONDUCT AND INTERPRETATION OF DERMAL ABSORPTION DATA IN HUMAN RISK ASSESSMENT

John M Perkins
Global Leader Dermal Absorption
Human Health Assessment
Dow AgroSciences
Milton Park Abingdon, UK

Introduction

Dermal Absorption values are among the Critical End-points in the Commissions Review Report for compounds assessed under the 91/414/EC Directive. These absorption values can have a more significant impact on the Risk Assessment than the variability in the selection of the AOEL or the external exposure received by the worker.

There are well established International OECD Guidelines and Guidance (2004) on the conduct of dermal absorption studies. These documents contain all the information to facilitate the generation of regulatory acceptable dermal absorption data. However, no guidance is given on the interpretation of dermal absorption data which result in the Critical End-point values.

Guidance for the Interpretation of Dermal Absorption Data

Guidance on the interpretation is currently covered by an EU Guidance Document on Dermal Absorption, Sanco 222/2000 rev. 7, 19 March 2004. The guidance covers two scenarios:

1. Compounds with no dermal absorption studies or studies that are deemed not relevant.
2. Dermal absorption studies which meet the current OECD guidelines.

Specific aspects of these two scenarios are considered in detail below.

Compounds with No Dermal Absorption Studies or Studies that are Deemed Not Relevant

The EU guidance states that for ongoing evaluations where no measured data are available, **a default value of 10%** may still be used in the risk assessment by the Rapporteur Member State for the purpose of deciding on 'one safe use' in accordance of article 5(1) **unless there are clear indications that 10% would be unrealistically low (e.g. based on physical chemical properties of the active substance).**

In order to qualify for Default Values of 10% the following criteria have to be matched:

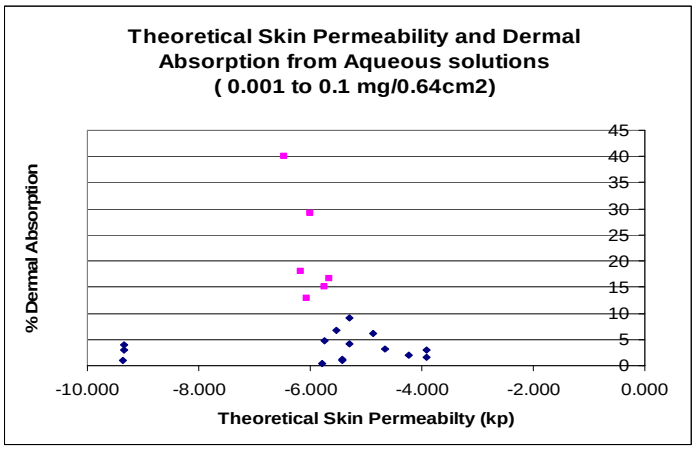
Molecular Weight > 500
and
Log K_{ow} <-1 or >4

Outside these criteria the proposed value is 100%. However, how many pesticides have a **"Molecular Weight > 500"??** The outcome is therefore a default value of 100%.

This presentation proposes that a more refined Predictive Option should be considered based on the Potts and Guy Model (1995) which considers Log K_{ow}, MW or MV and various constants to yield a theoretical Skin Permeability (K_p) value.

i.e. Log K_p = 0.71 Log K_{ow} – 0.0061MW - 6.3.

When this approach is applied to dilute aqueous solutions of commercial formulations the theoretical skin permeability can be compared to the experimentally derived dermal absorption value (e.g. % absorption).



Dermal Absorption in excess of 10% has only been found for theoretical Skin Permeability values of -5.6 to -6.5. ALL compounds have Molecular Weight <500.

Dermal Absorption Studies Meeting Current OECD Guidelines
The OECD documents recommend tape stripping the stratum corneum from epidermis/dermis. However the Sanco guideline states "the amount located in the skin is included as being absorbed based on expert judgment". No reference is made to tape stripping and/or exclusion of the SC from absorbed dose. The Scientific Committee on Plants, 30 April 2002, exclude Stratum Corneum from absorbed dose.

An example of the potential importance of the stratum corneum:

Location Stratum Corneum Tape Strips	**% of Dose**
2 -4	3.5
5-7	1.7
8-10	1
11-13	0.3

14-16	0.1
17-19	0.1
Total SC	6.8
Epidermis/Dermis	6.1
Receptor Fluid	2.9

Does the distribution in the stratum corneum suggest that this residue should be included in a total absorbed dose which is used to generate a daily (24hr) systemic exposure for comparison with an end-point expressed as mg/kg/day?

A related issue is the residue remaining at the application site in *in vivo* studies. The
EU Guidance Document States "in case the experiment is terminated before serial no detects….the amount in the skin should be considered as absorbed. Expert Judgment must be used." There is an on-going problem with the lack of expert judgment regarding **interpretation of the dose at the Application Site.**

An example of the potential importance of the application site was considered:

Sampling Interval (hrs)	**Matrix**	**Percentage**
48	Application site	38
72	Application site	35
72	Systemic Absorption	12
0-24	Systemic Absorption	8.23
24-48	Systemic Absorption	2.21
48-72	Systemic Absorption	1.73

Residues were detected in excreta at the end of the study, i.e. no serial non-detects. Should **the application site be added** to **give total absorption to be used in a per day risk assessment,** when only 5% of the application site is contributing to the systemic exposure residue over 48 to 72 hours?

Conclusions
The following areas require more detailed guidance in the interpretation of dermal data:
Impact of formulation type on dermal absorption, for example solvent- or water-based formulations and powders.
Impact of concentration and/or matrix on QSAR modelling.
Contribution of the Stratum Corneum and Application Site residues to the dermal absorption values and the resulting impact on Risk Assessment.

References

- OECD Guidance for the Testing of Chemicals. Guideline 427 and 428. 13 April 2004. Guidance Document for the Conduct of Dermal Absorption Studies. OECD Series on Testing and Assessment. No 28. 5th March 2004.

EU Guidance Document on Dermal Absorption, Sanco 222/2000 rev. 7, 19 March 2004.

- Opinion of the Scientific Committee of Plants on Commission Draft Guidance on Dermal Absorption (Doc SANCO/222/2000-rev 4. 2002).

- Potts, R.O., Guy R.H.1992. Predicting Skin Permeability. Pharmaceutical Research 12, 1628-1633.

DERMAL ABSORPTION IN RISK ASSESSMENT: THE USE OF RELATIVE ABSORPTION VERSUS PERMEATION COEFFICIENT (k_p)

Cees de Heer
TNO Quality of Life, Zeist, the Netherlands

Introduction

Dermal absorption of chemicals is often expressed as percentage of the dose coming in contact with the skin. The QSARs for dermal absorption, however, are all designed to predict kp-values. This raises the question in which way and for which exposure conditions the kp-value will provide a meaningful estimate for dermal absorption.

Modelling of skin absorption; theoretical considerations

Like the passage of substances through an artificial membrane, skin absorption is a passive, concentration-driven process that can be described by Fick's law. For description of *in vitro* dermal absorption often the following modification of this law is applied: $\mathbf{J_{SS} = k_p * C}$, where J_{SS} = steady state flux (after an initial lag time), k_p = permeation or permeability constant, and C = concentration of penetrant in donor fluid (concentration in receptor fluid assumed nil).

Fick's law is however only valid for homogenous membranes and under the condition of a constant concentration gradient over the skin in time. These conditions often do not reflect the situation of absorption of substances through the skin under realistic exposure conditions.

From testing for dermal absorption of chemical substances dissolved in different vehicles and formulations, it became clear that the matrix in which a substance is presented to the skin is decisive for the outcome of the experiment. Vehicles and substances may alter the barrier function of the skin, either increasing or decreasing its permeability. Furthermore, partitioning between vehicle and *stratum corneum* will vary with the nature of the vehicle.

Additionally, experimental data for e.g. nicotine and 2-butoxy ethanol, have shown that the steady state flux is not always linear with the concentration of a substance in an aqueous solution over the entire concentration range. The latter is a very basic assumption in the modelling of dermal absorption at infinite dose conditions.

The above means that the theoretical assumptions underlying the equations modelling dermal absorption may not always hold true under real life exposure conditions.

Relative absorption (% of dose) versus permeability coefficient (k_p):

	Relative absorption (% of dose)	**Permeability coefficient (k_p)**
Use in risk assessment	- finite exposure, such as spraying of plant protection products or biocides or dermal application of cosmetics	- infinite exposure, such as splash incidents - environmental exposures, such as exposure to contaminated water
Assumptions	- exposure duration realistic for worker or consumer, - amount of substance/cm^2 realistic for worker or consumer - vehicle/formulation realistic for worker or consumer	- concentration on the skin surface does not change during exposure - steady-state flux does not change during exposure period
Advantages	- experimental conditions can be directly matched to reflect worker exposure conditions - can be used for *in vivo* studies - skin depot can be taken into account by adding the percentage dose retained	- useful to estimate absorbed dose from different exposure conditions by extrapolation using computational modelling approach
Disadvantages	- extrapolation to alternative exposure conditions is difficult - no QSARs available - may result in overestimation or underestimation of dermal absorption	- may result in overestimation or underestimation of dermal absorption

Using k_p for finite dose dermal absorption estimates

k_p-values can be used to obtain a simple estimate of the maximum amount of absorption to be expected using the following formula:

$$A = k_p * C * t * SA$$

where A = amount absorbed (mg); k_p = permeability constant (cm h^{-1}); C = concentration (mg cm^{-3}); t = exposure time (h); and SA = exposed skin area (cm^2)

In this project, this calculation was applied to *in vitro* experimental data from a TNO-database and the EDETOX-database. For this, k_p-values were estimated with Potts & Guy QSAR (Potts & Guy, 1992) based on log P and molecular weight. Subsequently, the dermal absorption in the diffusion cell was calculated with the above formula and compared with the actual experimental results.

Results
The use of modeled k_p-values (derived from QSARs) to predict the dermal absorption in *in vitro* experiments may lead to both overestimation and underestimation of dermal absorption. However, in most cases a significant overestimation of the experimental absorption was observed. When comparing relative dermal absorption data measured *in vitro* to calculations on the basis of modeled k_p-values, overestimations (of actual absorption) up to ca. 1000 times the measured values were observed and underestimations up to a factor 3.

Underestimation of actual absorption may occur when:
- sink conditions are not ideal, e.g. when the compound accumulates in the dermis, leading to a reduced flux
- presence of skin depot, which may become systemically available, is not taken into account
- test compound or formulations decrease skin barrier function
- absorption of compound in vehicle has a clearly higher skin penetration than neat compound (e.g. nicotine, 2-BE)

Overestimation may be due to:
- Test compound or formulations increase skin barrier
- Lag-time is significant compared to exposure time
- Exhaustion of the donor compartment

Conclusions
Use of k_p-values (infinite dose, using water as vehicle) may lead to over- or underestimation of actual absorption due to e.g. influence of test compound or vehicle on barrier function

Direct use of only k_p-values to calculate absorption at finite doses does not produce realistic results

A better 'extrapolation' of k_p values to finite dose conditions is a prerequisite for broad application of the k_p in risk assessment.

Recommendations
Develop generic models (preferably requiring little to no experimental data) to extrapolate kp-values to finite doses

Investigate whether implicit and explicit assumptions for deriving kp-values hold true for a (large) number of model compounds

Develop QSARs for vehicles other than water (e.g. liquid formulations)

CONSUMER PRODUCTS - DERMAL EXPOSURE AND RISK ASSESSMENT

David McCready
The Dow Chemical Company, South Charleston, West Virginia, USA

This presentation focuses on the Dow approach for modeling dermal exposure to consumer products. It uses an efficient, tiered approach. Some practical risk assessment guidance and common areas with the EC approach were discussed.

A summary of the tiered modeling approach for dermal exposure:
- Exposure assessment is performed in steps.
- Dermal absorption is the key variable in the tiered approach.
- One iterates from simple to refined analysis. Low effort/cost to high effort/cost.
- Tiered approach has common areas with EC Guidance Document on Dermal Absorption for pesticide products.
- Dow may use experimental data or QSARs.

A description of the tiered modeling approach, as illustrated in Figure 1:
- Tier 1 is a screening analysis that requires limited information. Makes conservative assumptions, such as 100% absorption of the substance. Worst case estimate of the potential exposure.
- Tier 2 uses more realistic assumptions. Steady-state dermal uptake equation in a spreadsheet. Use skin permeability from experiment or QSAR.
- Tier 3 refines the penetration and uptake by using a non-steady state model. Use PROMISE© or ConsExpo models.
- Tier 4 physiologically based pharmacokinetic (PBPK) model.

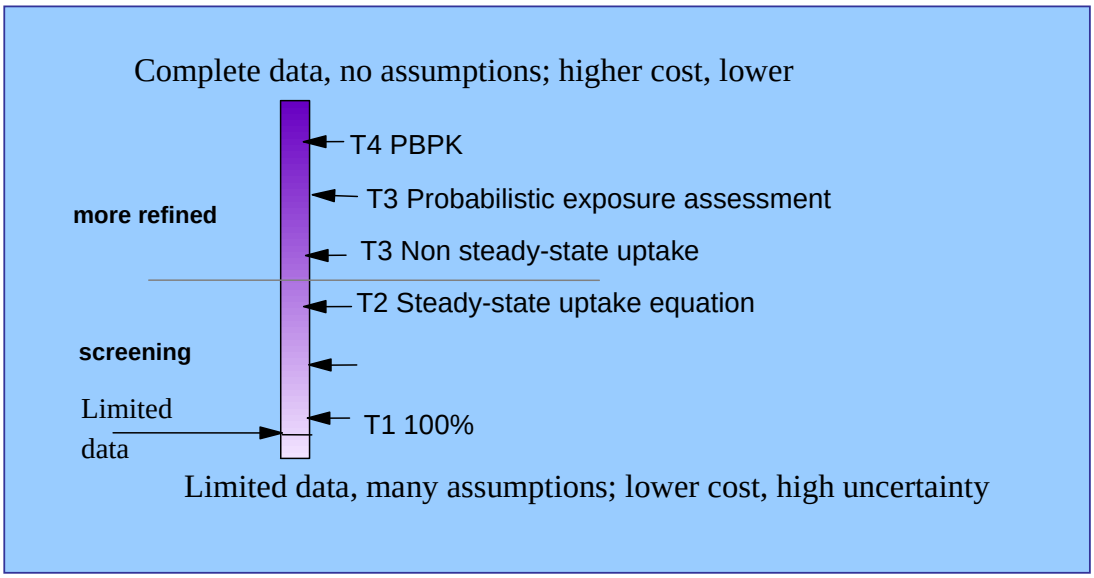
The following issues were discussed:
- Why Evaluate Consumer Exposure?
- Typical Steps in an Assessment
- Dermal Exposure Scenarios
- Typical Products & Substances Evaluated
- Exposure Scenario Development
- Many Products? Which to Evaluate First ?
- Risk or Hazard Assessment Steps
- EC Guidance Document on Dermal Absorption, March 2004
- Other important variables; contact time and surface area.

Some summary issues:
- To reduce effort – use previous studies, highest exposure product/scenario, use a tiered approach.
- Skin permeability is a primary variable. Use experimental data or QSAR.
- Applicability domain for experimental data or QSAR should match product.
- If use 100% dermal uptake, must refine other variables.
- Library of exposure factors is helpful.
- Substance toxicity may determine the tier used.

References

1. Organization for Economic Co-operation and Development (OECD), Guidance Document for the Conduct of Skin Absorption Studies, Series on Testing and Assessment, No. 28, 2004.
2. American Solvents Council, The PROMISE Model page, Available at http://www.americansolventscouncil.com/resources/promise.asp/; accessed on Feb. 23, 2004.
3. RIVM (National Institute of Public Health and the Environment), ConsExpo 4.0 model (Consumer Exposure and Uptake Models), Bilthoven, Netherlands; http://www.rivm.nl/en/milieu/risicos_stoffen/ConsExpo.jsp; accessed on August 11, 2005a.

Figure 1. *An Illustration of the Tiered Approach*



APPENDIX 1

Statements for Discussion in the Workshop

Global CEM Net Report of the Workshop no. 1 on
"*Dermal Transfer and Penetration Algorithms*", Intra (Italy), 20-21 June 2005

ISSUE 1: There is a disconnect between exposure assessment data collection and dermal absorption calculations. How can this be improved?

1. How can we proceed to estimate uptake when exposure measurements are generally only reported in terms of mass on skin when models of permeation are framed in terms of concentration of chemical applied to the skin?

2. Different exposure measurement methods, for example, interception and removal techniques, produce measurements in the same units but they do not measure the same thing! How should we ensure greater comparability in exposure measurement data?

3. Currently available methods to quantify outcome of dermal exposure provide a lot of 'noise' in view of uptake by skin. Estimates of mass, loading and concentration cannot be interpreted. State-of-the-art knowledge on uptake of chemicals by skin only needs adequate estimates of skin *surface exposed* and *exposure duration*.

4. There can be considerable spatial heterogeneity in the distribution of a chemical over the body region, with in some instances most of the chemical being accumulated over a small fraction of the body's surface area. If dermal absorption (expressed as a percentage of the available dose) varies with the mass loading of the skin, then how is the systemic dose determined from a single value representing the total mass of chemical residing on skin?

5. What is the effect of non-uniform distribution of chemical on dermal absorption?

6. There are clear indications for substantial temporal and spatial variability of all exposure parameters. These should be addressed in uptake studies to remain the driver for development of quantitative dermal exposure methods.

7. Extrapolating data from <u>large</u> applied doses to <u>small</u> applied doses
   - Is absorbed dose $\propto$ to applied dose?
   - Stated differently, is % absorption constant?

ISSUE 2: Effect of vehicles or matrices. Extrapolating from one vehicle (often water) to another (e.g. formulations)

8. The vehicle or matrix, in which a chemical contacts the skin, is a significant determinant of the resulting exposure. The level of uptake from one vehicle cannot be assumed to be predictive of that from a different matrix.

9. The permeability coefficient (Kp) of a chemical from aqueous solution can be predicted with some reliability from its physicochemical properties. Caution, and additional information, however, is required when using Kp to estimate risk (i.e., a quantitative determination of exposure) following dermal contact.

10. Extrapolating data from water to non-aqueous liquids
    - Can we use data measured from water to estimate absorption from non-aqueous liquids?

- How?

11. To what extent are models based on aqueous solutions adequate? Are theoretical models and appropriate QSPeRs a realistic prospect in the short to medium term? If not then how should we proceed?

ISSUE 3: How should the skin reservoir be handled in risk assessment?

12. The skin can act as a reservoir for a chemical so that absorption can continue long after exposure to the surface of the skin has ended. In these circumstances, the systemic dose on any particular day is a composite function of the exposure processes integrated over the current and previous days. In these circumstances, the capacity of the skin to act as a reservoir will lead to a smoothing of the exposure process. Therefore, single day exposure assessments (whether deterministic or probabilistic) have a tendency to overestimate variation in systemic exposure with a corresponding overestimation of risk.

13. Do we fully understand what happens when the skin is exposed to chemicals? How does a chemical mixture interact with the skin contaminant layer and how well do we capture these processes in models that are used to predict uptake?

14. Should contributions from the skin reservoir be considered?
    - At the end of the exposure, is the amount of chemical in the skin significant?
    - Does the chemical in skin at the end of the exposure contribute to health risk?

ISSUE 4: Almost no reliable measurements (and measurement methods) of bioavailability

15. Methods for the determination of local (i.e., skin) bioavailability of a chemical contacting the skin have not been optimized. Quantitative estimates are possible from both *in vitro* and *in vivo* experiments, but a validated, relevant and safe "gold standard" remains an elusive objective.

ISSUE 5: Estimates of uncertainty

16. Uncertainty in dermal absorption, expressed as either percentage penetration or as permeability coefficients will vary considerably depending upon the source data. For probabilistic exposure assessments to be viable there is an urgent need to define default uncertainty factors for animal-to-human, vehicle-to-vehicle and *in vitro* to *in vivo* extrapolations.

APPENDIX 2

Dermal Transfer and Penetration Algorithms (prior to Global CEM Net Workshop, June 20-21, 2005, Intra (Italy) White Paper)

Global CEM Net Report of the Workshop no. 1 on "*Dermal Transfer and Penetration Algorithms*", Intra (Italy), 20-21 June 2005

The purpose of this working paper is to provide context for the discussions in Workshop 1 of the Global Net meeting in June 2005. The text is almost in full currently identical to a white paper for a workshop at the OEESC-2005 Stockholm conference on skin issues.

Some recent activities

Recent studies, specifically the RISKOFDERM project, have established a large database of dermal exposure levels of chemicals in several occupational use scenarios (Ann. Occup. Hyg., 48/3 (2004)). The methods used to obtain these measurements were based on the OECD Guidance Document OCDE/GD (97)/148. Data were obtained over (part of) a work shift using state-of-the-art methodology that assessed the potential dermal exposure. This approach provides estimates of the mass of contaminant chemical that may be available to be taken up into the body, but does not take account of the protective effect of clothing or the mass of chemical that is likely to be taken up into the body. Estimation of uptake of chemicals through the skin is most relevant for risk assessment where an evaluation of systemic exposures is essential.

Other recently completed research, for example the EDETOX project (http://www.ncl.ac.uk/edetox/index.html), has focused on evaluation of permeation of chemicals through the skin. These studies have used both *in vitro* and *in vivo* assessment methodologies to assess the dermal uptake of chemicals. However, it is impractical to measure dermal permeation for the many thousands of industrial chemicals in use today. An alternative approach that has been proposed is to use predictions of steady-state permeation from statistically derived relationships between physical-chemical properties and the permeability coefficient of representative chemicals, relationships known as QSARs (quantitative structure-activity relationships).

In 2004 the European Chemical Industry Council (CEFIC) sponsored a scientific workshop to discuss skin permeation measurement methods (http://www.iom-world.org/news/ppworkshop.php). The workshop discussed, amongst others, the use of QSAR for risk assessment purposes. The data currently used for QSARs are obtained from "infinite dose" *in vitro* absorption studies, i.e. there is no limit to the amount of permeating chemical. Such studies determine the maximum flux (for the applied concentration), and from that flux a permeability coefficient k_p is calculated. The permeability coefficients for a set of chemicals are related by QSARs to physical-chemical properties such as octanol-water partition coefficient and molecular weight. However, realistic risk assessment scenarios usually correspond to "finite dose" conditions, plus current exposure assessment methods do not measure the concentration of contaminant chemicals.

The main immediate need identified by the CEFIC workshop was to establish the link between finite and infinite dose experiments, thus linking the QSAR derived information with the inputs required for risk assessment. The linkage between finite and infinite dose experiments relies on mathematical modelling and the associated relevant and reliable experimental data. These techniques enable a sound theoretical basis to be used in the interpretation of the data, and this should improve the reliability of parameters calculated from experimental data. The models also should enable extrapolation to predict absorption under different dosing conditions.

Up till now, there has been surprisingly little interaction between the researchers involved with occupational dermal exposure assessment and those researchers working on dermal permeation. This may be an important reason why there is a mismatch between the external exposure data obtained in

the field or through modelling attempts, even after correction for clothing penetration, and experimental or QSAR data on dermal permeation.

Dermal exposure

Humans are dermally exposed to environmental contaminants via three media of exposure water, soil, and air and as pure chemicals or mixtures in occupational settings. The site of dermal exposure is directly related to the activity being performed at the time of exposure. Several factors can influence dermal exposure during activities. These include:

- reduction or increases in the chemical contact with skin due to normal clothing;
- protective clothing and gloves worn by workers and the amount of protection they offer;
- individual differences in dermal exposure due to differing degrees of speed, care, and dexterity in performing work;
- variance in the amount of material available for dermal absorption due to actions such as wiping the affected area with the hand;
- variances in the penetrability of the skin in different parts of the body;
- individual variability in regards to skin penetrability due to age and skin condition, such as disease and thickness of the stratum corneum; and
- the matrix of the chemical contaminant, solid, liquid, or vapor.

Dermal exposure is defined as the process of contact between an agent and skin at an exposure surface over an exposure period. The (target) exposure surface in view of the dermal route is the skin contaminant layer (SCL) compartment, i.e. the compartment on top of the stratum corneum of the human skin, and is formed by sebum lipids, sweat and additional water from transepidermal water loss, rest products from cornification and unshed corneocytes, and is given by its three dimensional volume.

Parameters of the result of contact are: dermal exposure mass, i.e. the mass of agent present in the contact volume; dermal exposure loading, i.e. exposure mass divided by the skin surface area where an agent is present; dermal exposure concentration, i.e. exposure mass divided by the exposure volume (SCL) or the exposure mass divided by the mass contained in the SCL.

The current dermal exposure assessment methodology should be improved so that biologically relevant data are to be collected. The current methodology is mainly based on assessing total exposure mass. Measurement methods for dermal exposure assessment, i.e. to identify and quantify an agent, can be grouped according to three major principles:

- sampling by interception of agent mass transport towards clothing and/or skin by the use of collection media (pads) placed at the skin surface or replacing (work) clothing during the sampling time followed by Detection, e.g., chemical analysis of extracts from the collection matrix;
- sampling by removal of the agent mass from the skin surface (SCL) at any given time or the end of the sampling period (by wash liquid, wipe fabrics, etc.), followed by detection in the collection matrix;
- direct assessment by *in situ* detection of the agent or a tracer at the skin surface, e.g. by image acquisition and processing systems, at a given time.

Since *in situ* techniques also determine the surface areas actually exposed, the results also indicate exposure loading of the SCL, whereas the results of removal techniques can be used to estimate exposure loading of the skin surface.

Mass transport processes can be divided into processes towards the clothing and skin compartments and processes from clothing and skin compartments. The latter are subdivided into two pathways: from the skin contaminant layer into the skin by uptake, and transport from the skin contaminant layer to other compartments by removal, resuspension or evaporation. High or low transport rates will bias the results obtained by different sampling methods. Low transport rates allow use of removal and in situ detection techniques applied immediately before decontamination to adequately estimate the level of contamination of the skin contaminant layer relevant for uptake. If the removal-resuspension / evaporation rate is low, but uptake rate is high, an interception sampler or an in situ-direct technique would give a good measure of dermal uptake. If the removal-resuspension/evaporation rate is high and uptake rate is low, an interception sampler (assumed to have a better retention performance compared to skin) would greatly overestimate uptake. In this case biological monitoring, being a non-route specific method for uptake, would be preferable, and also in the cases that both transport rates are high. Since the results obtained by different sampling principles are influenced by a range of mass transport processes and may have to be extrapolated beyond the sampled contact volume, all sampling methods are faced with fundamental problems, such as:

- interception and retention characteristics of interception techniques differ from real skin or clothing;
- removal methods, e.g. tape stripping, solvent washing, and use of surfactants, may influence the characteristics of the skin; they may also be of limited use for repeated sampling;
- removal techniques, e.g. skin washing, are not appropriate for all body parts;
- extrapolation from small areas sampled, e.g. pads (patches) or skin strips, to the whole exposed area can introduce substantial errors;
- Behavior of a (fluorescent) tracer introduced in the mass transport when using in situ-techniques may differ from the behavior of the substances of interest.

As indicated, the total mass measured may be a poor surrogate for the uptake, either since the mass of chemicals on the skin is not all available for uptake or is spread very unevenly on the skin. It would be more relevant to measure the exposure using a sampler that was a closer mimic of the skin, just as for inhalation exposure respirable dust sampling can be used to select the biologically relevant exposure to dust. Progress has been made in developing a prototype diffusive dermal sampler based on an adsorbent sandwiched between a semi-permeable barrier membrane and an impervious backing. Further development of this type of sampler may in the longer term offer a more appropriate measurement method.

Information about soil or sediment adherence, dermal transfer from surfaces, contact rates, and frequencies for important exposure scenarios is very limited. Only a few studies have been conducted to better characterize dermal contact and chemical transfer to the skin. These studies have focused on chemical release from sediments, sediment adherence to skin, and residue transfer from treated surfaces to skin. More studies are needed to better characterize activities associated with these environmental exposures.

Surface contact occurs when the skin comes into contact with a contaminated surface and chemical residue is transferred to the skin. This may contribute to oral exposure if chemical residues on the

hands are transferred to the mouth or transferred from the hands to food. One major step to estimate dermal contact accurately is to better define the activity being performed at the time of exposure. If the activity patterns of humans were better characterized, the uncertainty of the dermal exposure characterization process would be greatly reduced.

Dermal absorption

The physiology and biochemistry of the skin can account for much of the variability associated with dermal absorption of substances. The three routes of entry through the skin are the stratum corneum, the sweat duct, and the hair follicle.

The amount of chemical coverage on the skin surface can influence the amount of dermal absorption. Chemical coverage of the skin may be incomplete or exceed the exposed skin surface area by piling up on itself. Likewise the transfer efficiency from a contaminated surface to the skin or liquid solution may be highly variable due to the nature and extent of the contact or the deposition of chemical residue due to evaporation of the liquid.

Quantitative exposure assessments for contaminants in water and air are based on the use of a permeability constant (K_p in cm/hr), which is a measure of the rate of penetration into the skin. K_p is usually measured in the laboratory from *in vitro* studies at steady state (infinite dose experiments). For exposure to soil, percutaneous absorption is usually expressed as the fraction of the applied dose absorbed from both *in vivo* and *in vitro* studies. For applications of soil containing equal concentrations of a contaminant, the amount of soil that adheres to the skin determines the amount of contaminant absorbed. Many of the permeability coefficients are based on predictive methods that commonly use octanol-water partition coefficients (P_{ow}) and molecular weight due to a lack of experimentally derived permeability coefficients for many chemicals. Most experimentally derived permeability coefficients are determined using the pure chemical deposited onto skin in a volatile solvent (e.g., acetone or ethanol) or the chemical in an aqueous solution. A number of factors may influence dermal absorption estimation such as physical and chemical characteristics of the contaminant (including factors such as corrosivity), matrix composition, physiological characteristics of the skin (including anatomical site or species), amount of surface area contact, and rate and mechanism of absorption.

Quantification of percutaneous penetration is an essential step in reducing the uncertainty of dermal risk assessment. Generally, if no quantitative absorption data are available for a substance it is assumed that 100% of the material applied to the skin surface is available systemically. This is an extremely conservative assumption, yet necessary due to the lack of data concerning absorption rates for chemicals.

Rates of permeation of chemicals cannot be precisely measured by analysis of absorbed material in excreta. Therefore, permeability constants are difficult to determine by those *in vivo* techniques, although *in vivo* Kp assessments can be improved by blood sampling, followed by pharmacokinetic analysis. *In vitro* techniques can be used to provide fast, direct measurements of flux and permeability constants (Kp) in human skin. In addition, factors affecting dermal absorption from various matrices (soil, water, oil etc.) can be controlled in *in vitro* studies. The most relevant percutaneous penetration data comes from human volunteer studies, but these data are rare. Costs and ethical constraints frequently rule out the testing of toxic compounds in humans. This necessitates the use of *in vivo* animal or *in vitro* methods which requires extrapolation of the results to those expected in humans.

Predicting dermal absorption with mathematical modelling

An approach for validating *in vitro* techniques is to use *in vitro* derived parameters of skin barrier function (i.e., the permeability coefficient and partition coefficient) in mathematical model representing *in vivo* absorption. In this approach, experimental variables in the *in vitro* and *in vivo* studies do not have to be identical as long as differences are described in the mathematical model.

Mathematical modelling can be used to describe the dermal absorption process by applying conservation of mass equations. Mathematical models that are mechanistically based require physicochemical parameters for the absorbing chemicals (e.g., diffusion coefficients and partition coefficients or parameters derived from these like the permeability coefficient). Depending on the situation that these mathematical models are describing, they will also include the volume of the vehicle, blood flow rates and so on. If the physicochemical parameters for a given compound are available, then these models can be used to describe dermal absorption for situations other than those used in the experiments in which the physicochemical properties (e.g., permeability coefficient and partition coefficient) were measured. For example, the mathematical model can use steady-state *in vitro* measurements to predict unsteady-state finite dose *in vivo* measurements, at least when lag time or equivalent information is known. These models are distinctive from QSAR models in that QSAR models are used to relate chemical structure to the physicochemical parameters that are important to dermal absorption, i.e., permeability coefficients and partition coefficients. For example, the permeability coefficient is a measure of a chemical's diffusivity and solubility in the skin layers relative to the vehicle. Diffusivity is known to vary with molecular size. Small molecules diffuse faster than big molecules. Solubility depends on how similar (or different) the chemical is to the skin layers (i.e., the stratum corneum and the viable epidermis) compared to the vehicle that the chemical is in when it is presented to the skin. QSAR models are used to estimate/predict the physicochemical properties needed in the dermal absorption model The mathematical model lets you use measurements made in one type of experiment to estimate dermal absorption in a different exposure scenario.

Predicting dermal absorption with QSAR techniques

Penetration of chemicals through the skin can be described as diffusion through a pseudo-homogenous membrane. This can be described using Fick's first law that states, "the flux of the penetrating chemical at a location within the membrane barrier is proportional to the membrane diffusion coefficient and the concentration gradient at that position". When skin is exposed to a chemical, chemical penetration through the stratum corneum will be initially rapid and slow as it satisfies the capacity of the stratum corneum for the chemical. At this point absorption is unsteady and the chemical has not reached the systemic circulation. The chemical will then reach the systemic circulation and, if exposure continues, the concentration gradient through the skin will become constant. At this point absorption has reached steady state meaning the mass of chemical entering and leaving the skin are constant. A simplified mathematical model has been developed that successfully estimates dermal absorption from infinite dose aqueous solutions by taking into account both the non-steady state and steady state period of absorption. This model can be represented using 2 algebraic equations, one for the non-steady state absorption period and one for the steady state absorption period. In combination with QSAR models for the permeability coefficient and partition coefficient, this model can predict dermal absorption for chemicals that have not been studied. These simple equations have provided reasonable estimates of *in vitro* and *in vivo* data. It should be mentioned that nearly all of the QSAR equations for estimating permeability coefficients are

restricted to aqueous solutions. This is because permeability coefficients are vehicle dependent and nearly all of the data are from aqueous vehicles.

The first type of equation/model is used to estimate the physicochemical parameters that characterize dermal absorption, i.e., permeability coefficients, partition coefficients, diffusion coefficients, lag times, and so on. The second type of equation/model uses these physicochemical parameters in an equation to estimate dermal absorption.

Most of the QSAR equations are the first type. They are structure-activity based equations designed to estimate chemical properties in skin. Many of the QSAR equations for skin estimate "steady-state" permeability coefficients from aqueous vehicles. However, there are QSAR equations for estimating partition coefficients between the stratum corneum (or sometimes skin) and aqueous vehicles and for estimating effective diffusion coefficients in the skin.

Dermal absorption is usually estimated using the second type of equation, which is some sort of mass balance model that uses parameter estimates, perhaps from a QSAR equation. These equations can and have been written to account for lag time and the material remaining in the skin as well as for concentrations changing in the vehicle applied to skin. For example:

*Cumulative mass absorbed = permeability coefficient * concentration * exposure time (Eq. 1)*

This equation comes from a steady-state mass balance. The permeability coefficient may be estimated using QSAR, but this does not make the above equation itself a QSAR equation. Eq. 1 does not account for lag time. If the exposure time is taken to be the time until the exposure ends, then Eq. 1 also does not account for material that will still be in the skin when the exposure ends. However, these flaws are not part of the QSAR equation; they are flaws of the mass balance equation.

Mass balance equations are not restricted to steady state and can be derived to include absorption of chemical in the skin at the end of the exposure. This is the case in the equations recommended for estimating dermal absorption from contaminated water. The recommended mass balance based equations (i.e., the second type of equation) use lag time (really an estimate of the diffusion coefficient) and permeability coefficient. Estimates of lag time and the permeability coefficient are calculated using QSAR equations. The permeability coefficient can be estimated with the same equation.

There are a very few equations of a third type, which estimate dermal absorption directly using only structure-activity parameters, but these equations can only be used for the situations in which they were derived.

The chief advantage of the strategy of estimating the physicochemical parameters by QSAR and then incorporating these into mass balance equations is that it can be used for a wide variety of exposure scenarios, provided we have the appropriate physicochemical data.

The chief problem right now is that permeability coefficients (or alternatively, partition coefficients) are not available for non-aqueous vehicles and we do not know how permeability coefficients are affected by dermal absorption of multiple compounds at the same time.

Mind set and goals of the workshop

The purpose of this workshop was:

> *1. To survey and discuss the general state-of-the-science of the methodology for assessing dermal penetration.*
> *2. To identify recommendations for next steps in modelling dermal exposure to consumer products and then prioritize the recommendations for future research.*

The focus of the workshop is not on specific substances but on the identification and development of general modelling constructs capable of describing the relevant factors for the multitude of substances impacting and penetrating human skin.

The expected duties of and opportunities for the participants have been to:

> *1. Provide feedback and material to the workshop report to be drafted by the moderator before, discussed during and finalized after the Workshop.*
> *2. Formally or informally present relevant research that you have done or have specific knowledge of, relative to these two general areas of study.*

This was done by covering at least the following issues:

a. How to use JRC EIS Chemrisks "ExpoData" to help drive research needs?
b. How to address regulatory policy, specifically EC Guidance Document on Dermal Absorption dated 19 March 2004?
c. Considering the tiered approach to modelling dermal exposure, what sort of approach is necessary? A simple approach to screen many chemicals or a refined approach to estimate chemical specific dermal exposure?
d. What are the data needs for modelling dermal exposure to consumer products?
e. Which dermal exposure models are readily available and documented (either separate or integrated)?
f. How to address dermal uptake (experimental and model) uncertainty and variability?
g. How to "compare or corroborate" model predictions to experimental results?
h. What are the top priority dermal exposure chemicals/products/scenarios?

APPENDIX 3

Agenda for Global CEM Net Workshop no. 1 on "Dermal Transfer and Penetration Algorithms" June 20-21, 2005, Intra (Italy)

Global CEM Net Report of the Workshop no. 1 on
"*Dermal Transfer and Penetration Algorithms*", Intra (Italy), 20-21 June 2005

Day 1 (June 20, 2005)

Moderator: *Joop J. van Hemmen*
Rapporteur: *Katinka E. van der Jagt*

1. General

- Introduction to the workshop and Global Consumer Exposure Modeling Network *(Stylianos Kephalopoulos*)
- Results of the OEESC-2005 workshop (June 12, 2005 in Stockholm) *(Joop J. van Hemmen)*

2. Methodology

Exposure modeling

- The effect of spatial and temporal variations in exposure on dermal absorption (*Nick Warren*)
- A users experience with the dermal modules of ConsExpo and PROMISE, and a vision for future models (*Tip Tyler)*

Skin absorption

In vivo

- Efforts to harmonize dermal exposure assessment methods *(Mike Dellarco)*

In vitro

- Dermal absorption data generated by in vitro methods. Why do they deviate from QSAR predictions? *(Faith Williams)*
- The assessment of the dermal bioavailability of chemicals by using appropriate in vitro methods *(Winfried Steiling)*

(Q)SARs

- QSAR, REACH and the prediction of skin permeability *(Mark Cronin)*
- Modelling skin penetration: QSARs and mathematical models *(Sean Corish)*
- The possibilities to make more reliable risk estimates of exposure to substances via the dermal route (*Wil ten Berge)*

PBPK

- Effect of skin model form on PBPK performance *(John C. Kissel)*
- Dermal exposure to soils and solvent deposited solids: is absorption proportional to the exposed dose (*Annettte L. Bunge)*

3. Use of skin absorption data in human risk assessment

Cosmetics

- Skin absorption data in human risk assessment *(Walter Diembeck)*

Pesticides/biocides

- How dermal bioavailability data is used in human risk assessment of pesticides/biocides *(John Ross)*
- Conduct and interpretation of dermal absorption data in human risk assessment *(John Perkins)*

General chemicals

- Dermal absorption in risk assessment: the use of relative absorption versus permeation coefficient (k_p) (*Cees de Heer)*

Consumer products

- Human risk assessment for dermal exposure to consumer products *(David McCready)*

Day 2 (June 21, 2005)

1. Break-out groups

Use of Kp in risk assessment (algorithms)
[Chair: Annette L. Bunge; Rapporteur: Mark Cronin]

Integrating exposure and absorption (how can it be done in modelling approaches?)
[Chair: John Ross; Rapporteur: Cees de Heer]

2. Plenary session

Reports and conclusions from break-out groups

3. Conclusions and recommendations

- Are all issues covered and what can be recommended?

- Directions for future research

- Some specific questions were posed in advance:

a. How to use JRC EIS Chemrisks "ExpoData" to help drive research needs?
b. How to address regulatory policy, specifically EC Guidance Document on Dermal Absorption dated 19 March 2004?
c. Considering the tiered approach to modelling dermal exposure, what sort of approach is necessary? A simple approach to screen many chemicals or a refined approach to estimate chemical specific dermal exposure?
d. What are the data needs for modelling dermal exposure to consumer products?
e. Which dermal exposure models are readily available and documented (either separate or integrated)?
f. How to address dermal uptake (experimental and model) uncertainty and variability?
g. How to "compare or corroborate" model predictions to experimental results?
h. What are the top priority dermal exposure chemicals/products/scenarios?

[Chair: Han van de Sandt; co-chair: David McCready]

APPENDIX 4

List of Participants to the Global CEM Net Workshop no.1 on "Dermal Transfer and Penetration Algorithms"

WORKSHOP PARTICIPANTS

ten Berge, Wil, Santoxar, Westervoort, The Netherlands
Bunge, Annette L., Colorado School of Mines, Golden, CO, USA
Corish, Sean, University of Dublin, Dublin, Ireland
Cronin, Mark, Liverpool John Moores University, Liverpool, England (***Global CEM Net Workshop no. 1 Rapporteur break-out session 1***)
Dellarco, Mike, US-EPA, Washington, DC, USA
Diembeck, Walter, Beiersdorf A.G., Hamburg, Germany
de Heer, Cees, TNO Quality of Life, Zeist, The Netherlands (***Global CEM Net Workshop no. 1 Rapporteur break-out session 2***)
van Hemmen, Joop J., TNO Chemistry, Zeist, The Netherlands (***Global CEM Net Workshop no. 1 Moderator***)
van der Jagt, Katinka E., EMEA, London, England (***Global CEM Net Workshop no.1 Rapporteur***)
Kephalopoulos, Stylianos, JRC/IHCP/PCE, Ispra, Italy (***Global CEM Net Co-ordinator***)
Kissel, John, University of Washington, Seattle, WA, USA
McCready, David, The Dow Chemical Company, South Charleston, West Virginia, USA
Papameletiou, Demosthenes, JRC, IHCP, PCE, Ispra, Italy (*only for a small part of the time*)
Patlewiecz, Grace, JRC/IHCP/ECB, Ispra, Italy (*only first day*)
Perkins, John, Dow Agroscience Ltd., Milton Park Abingdon, England
Ross, John, Infoscientific, Carmichael, CA, USA
van de Sandt, Han, TNO Chemistry, Zeist, The Netherlands (***Global CEM Net Workshop no.1 Chair of Concluding Session***)
Steiling, Wilfried, Henkel KGaA, Düsseldorf, Germany
Tyler, Tip, Health Studies Management & Consulting, Annandale, VA, USA
Warren, Nick, Health and Safety Laboratory, Buxton, England
Williams, Faith, University of Newcastle, Newcastle, England
Worth, Andrew, JRC/IHCP/ECB, Ispra, Italy *(only first day*)

Stylianos Kephalopoulos
European Commission
Joint Research Centre
Physical and Chemical Exposure Unit
21020 ISPRA (VA)
ITALY
Tel: +39 0332 78 9871
Fax: +39 0332 78 5867
Email: stylianos.kephalopoulos@jrc.it

Joop Van Hemmen
TNO
PO BOX 360 3700 AY
Zeist
The Netherlands
Phone: +31 30 6944 913
Email: vanhemmen@chemie.tno.nl

Katinka Van der Jagt
EMEA - Veterinary
London
United Kingdom
Phone: +44 207 523 7232
Fax: +44 207 418 8447
Katinka.VanDerJagt@emea.eu.int

Annette Bunge
Colorado School of Mines,
Chemical Engineering Dept.
1613 Illinois St., Ste. 451
Golden, CO 80401
United States
Phone: +1 303 273 3722
Fax: +1 303 273 3730
Email: abunge@mines.edu

John Corish
Trinity College Dublin
University of Dublin, College Green
Dublin, 2
Ireland
Phone: +353 1 608 1776
Fax: +353 1 671 2826
Email: jcorish@tcd.ie

Mark Timothy David Cronin
Liverpool John Moores University
School of Pharmacy and Chemistry
Byrom Street
Liverpool, L3 3AF
England
Phone: +44 151 231 2066
Fax: +44 151 231 2170
Email: m.t.cronin@livjm.ac.uk

Cornelis/Cees de Heer
TNO Quality of Life
PO BOX 360
3700 AJ Zeist
The Netherlands
Phone: +31 30 6944 556
Fax: +31 30 6944 926
Email: deHeer@chemie.tno.nl

Michael Dellarco
US Environmental Protection Agency
(NCEA 8623 N) 1200 Pennsylvania AVE NW
Washington, DC 20460
United States
Phone: +1 202 564 3239
Fax: +1 202 564 2018
Email: Dellarco.mike@epa.gov

Walter Diembeck
Beiersdorf
Vinnastr. 48
Hamburg 22529
Germany
Phone: +49 40 4909 2662
Fax: +49 40 4909 3589
Email: walter.diembeck@beiersdorf.com

John Kissel
U OF Washington
BOX 354695
Seattle, WA 98195-4695
United States
Phone: +1 206 543 5111
Fax: +1 206 543 8123
Email: jkissel@u.washington.edu

David McCready
The Dow Chemical Company
3200 Kanawa Turnpike,
Bld 2000/Room 2416
South Charleston WV 25303
United States
Phone: +1 304 747 3714
Fax: +1 304 747 5963
Email: dmccready@dow.com

Demosthenes Papameletiou
European Commission
Joint Research Centre
Physical and Chemical Exposure Unit
21020 ISPRA (VA)
ITALY
Tel: +39 0332 78 5282
Fax: +39 0332 78 9453
Email: demosthenes.papameletiou@jrc.it

Patlewicz Grace
European Commission
Joint Research Centre
European Chemical Bureau
21020 ISPRA (VA)
ITALY
Tel: +39 0332 78 9566
Fax: +39 0332 78
Email: grace.patlewicz@jrc.it

John Perkins
Dow AgroSciences
2nd Floor, 3 Milton Park
Abingdon, Oxon
England
Phone: +44 1235 437 939
Fax: +44 1235 437 998
Email: jmperkins@dow.com

John Ross
Infoscientific.com, Inc.
5233 Marimoore Way
Carmichael, CA 95608
United States
Phone: +1 (916) 486-6100
Fax: +1 (916) 486-6181
Email: jhross@sbcglobal.net

Winfried Steiling
Senior-Manager Human Safety
Assessment
HENKEL KGaA, D-40191 Düsseldorf
VTF-Human Safety Assessment
Dr. Winfried Steiling
Phone: +49-211-797-9575
Fax: +49-211-798-8965
E-Mail: winfried.steiling@henkel.com

Willibrordus ten Berge
Retired from DSM
Wolter Visscherstraat 40
Westervoort
The Netherlands
Phone: +31 26 311 8921
Email: wtberge@planet.nl

Tipton Tyler
Health Studies Management & Consulting
4212 Wynnwood Drive
Annandale, VA22003
United States
Phone: +1 703 354 7306
Fax: +1 703 354 7301
Email: trtyler@att.net

Johannes Van de Sandt
TNO Quality of Life
Utrechtsewes 48
Zeist
The Netherlands
Phone: +31 30 69 44 619
Fax: +31 30 69 46 742
Email: vandesandt@chemie.tno.nl

Nick Warren
Health and Safety Lab.
Harpul Hill
Buxton Derbyshire
United Kingdom
Phone: +44 1298 218 442
Fax: +44 1298 218 471
Email: Nick.Warren@hsl.gov.uk

Faith Williams
University of Newcastle
Devonshire Building
Newcastle
United Kingdom
Phone: +44 191 246 4839
Fax: +44 191 246 4998
Email: f.m.williams@newcastle.ac.uk

Andrew Worth
European Commission
Joint Research Centre
European Chemical Bureau
21020 ISPRA (VA)
ITALY
Tel: +39 0332 78 9566
Fax: +39 0332 78
Email: andrew.worth@jrc.it

European Commission

EUR 22521 EN/1 – DG Joint Research Centre, Institute for Health and Consumer Protection
Title: Global Net on "Consumer Exposure Modelling": Report of Workshop no. 1 on "*Dermal transfer and penetration algorithms*"
Authors: *Stylianos Kephalopoulos, Joop J. van Hemmen, Katinka van der Jagt*
Luxembourg: Office for Official Publications of the European Communities
2006 – 98 pp. – 21 x 29,7 cm
EUR - Scientific and Technical Research series; ISSN 1018-5593
ISBN 92-79-03672-6B B

Abstract:

The purpose of the Global Net on "Consumer Exposure Modelling" Workshop no. 1 on "*Dermal transfer and penetration algorithms*" took place on 20-21 June 2005 in Intra (Italy) was:

> *1. To survey and discuss the general state-of-the-science of the methodology for assessing dermal penetration.*
> *2. To identify recommendations for next steps in modelling dermal exposure to consumer products and then prioritise the recommendations for future research.*

The focus of the workshop was not on specific substances but on the identification and development of general modelling constructs capable of describing the relevant factors for the multitude of substances impacting and penetrating human skin.

The outcome of the Workshop is summarized below:

After the presentations of the results of two break-out groups a general discussion took place which led to the following conclusions and recommendations.

1. In occupational situations, the skin contact time is often estimated on the basis of worst case considerations (e.g. 6-8 h per day). However many activities, such as mixing and loading, are generally performed within a much shorter time span. In addition, loading of the skin is not necessarily an instant process, but may occur over time.

2. The deposition of a substance is not homogeneous over the exposed skin area. The variability of the loading of the skin is likely to affect the skin absorption since relative skin absorption (% of dose) of a substance decreases with increasing dose.

3. In order to address points 1 and 2 in the risk assessment, there is a need for probabilistic exposure models. Dedicated studies should provide suitable data for these generic models. New studies may be needed to fill data gaps.

4. From a scientific point of view, the maximum flux should be used in preference to relative absorption in risk assessment. However, it is recognized that this approach may lead to overestimation of the actual skin absorption. QSARs may be used in the following tiered approach:
   - Tier 1 100% absorption
   - Tier 2 QSAR for max flux
   - Tier 3a in vitro testing using human (or pig) skin
   - Tier 3b in vitro testing using rat skin
   - Tier 4 in vivo test in rat (PBPK)
   - Tier 5 biomonitoring (PBPK)

   Guidance on the use of QSARs for regulatory purposes is considered necessary.

5. For further development of QSARs, databases containing measured and well-defined skin absorption data are of great importance. Evaluation of this existing data will allow for proper definition of the use of QSAR (e.g. applicability domain, dose levels, vehicles).

6. There is a need for generating data outside the present applicability domains ("unhappy domain"). Although it is recognized that human in vivo studies are the gold standard, standardized in vitro methodology is considered advantageous for cost-effective testing of substances with toxic or unknown properties.

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

LB- NA- 22521- EN/1- C

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

ISBN 92-79-03672-6

9 789279 036729

Publications Office
Publications.eu.int