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From dermal exposure to internal dose

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Exposure scenarios form an essential basis for chemical risk assessment reports under the new EU chemicals regulation REACH (Registration, Evaluation, Authorisation and restriction of Chemicals). In case the dermal route of exposure is predominant, information on both exposure and dermal bioavailability is necessary for a proper risk assessment. Various methodologies exist to measure dermal exposure, providing quantitative or semiquantitative information. Although these studies may provide very specific and relevant information, it should be realized that case by case in-depth exposure assessment would be a very expensive process. Dermal bioavailability data are most often obtained from *in vitro* studies or animal experiments. For the design of studies, which generate data relevant for chemical risk assessment, detailed information on the exposure conditions is crucial (skin surface exposed, exposure duration, dose and physical state of the chemical). Results from non-testing methods for skin absorption, such as (Q)SARs, have been used only to a very limited extent for regulatory purposes. Suggestions are made in order to extend the use these methods to dermal risk assessment of chemical substances, thereby improving the practicability of REACH.
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Introduction

Exposure scenarios form an essential basis for chemical risk assessment reports under the new EU chemicals regulation REACH (Registration, Evaluation, Authorisation and restriction of Chemicals), and generic exposure assessment tools generating scientifically justified and realistic exposure estimates would significantly increase cost-effectiveness of REACH. Routes of exposure include oral, inhalation and dermal, and various methodologies have been developed to estimate the amount of a substance coming in contact via these routes. In the case of dermal exposure, the site of contact poses a potential barrier to the substance. Therefore, data on dermal bioavailability are an integral part of risk assessment of substances/products to which man is predominantly exposed via the dermal route. Such data are generally generated experimentally (*in vitro* or *in vivo*) according to various guidelines prepared to harmonize study designs and evaluation of the results (OECD, 2004a, b, c; SCCP, 2006; EC, 2004). Special experimental techniques are increasingly used to accurately determine the location of the compound in the various layers of the skin. The detail of these data is in sharp contrast to that of most exposure estimates. This can partly be explained by the complexity and costs of such exposure studies, requiring a relatively large number of human subjects to study the scenario of interest and to select scenarios that represent typical exposure events. Selection of a single broad exposure scenario is frequently used in place of a field study, either during production/manufacturing and/or during actual use, to estimate dermal exposure. However, it should be realized that high-quality information on dermal exposure is crucial for designing proper testing strategies on skin bioavailability. Only by combining data on exposure and absorption, can predictions be obtained on internal dose, to be used for risk assessment purposes.

Dermal exposure

In line with the generic definition of exposure (Zartarian et al., 2005), dermal exposure has been defined as the process of contact between an agent and skin at an exposure surface over a period. The (target) exposure surface in view of the dermal route is the skin contaminant layer (SCL) compartment, that is, 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; the contact volume is equivalent to the volume of the skin contaminant layer, and

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for practical reasons, it is defined by the mass (kg) of all substances contained in this compartment), dermal exposure loading (i.e. dermal exposure mass divided by the skin surface area where an agent is present) and dermal exposure concentration (i.e. dermal exposure mass divided by the exposure volume of the SCL, or the exposure mass divided by the mass contained in the SCL) (Brouwer et al., 2005).

Humans are dermally exposed to chemicals (pure substances or mixtures) via the environment, as consumer or 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, including:

- Reduction or increase of 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 or cleaning the affected area; evaporation
- Variances in the barrier function of the skin in different parts of the body;
- Individual variability with regards to skin barrier function due to age and skin condition and
- The matrix of the chemical contaminant, solid, liquid or vapor.

The current methodology for measuring external exposure loading is mainly based on assessing total exposure mass (Van Hemmen and Brouwer, 1995; RISKOFDERM, 2004). Measurement methods for dermal exposure assessment, that is, to identify and quantify an agent, can be grouped according to three major principles:

- Sampling by interception of agent of mass transport toward 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, for example, 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 at the end of the sampling period (by wash liquid, wipe fabrics, tape stripping etc.) followed by detection in the collection matrix;
- Direct assessment by *in situ* detection of the agent or a tracer at the skin surface, for example, 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 toward the clothing and skin compartments and processes away from clothing and skin compartments. The latter are subdivided into the following two pathways: from the skin contaminant layer into the skin by uptake, and transport from the skin contaminant layer 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*-directed technique would give a good measure of dermal bioavailability. If the removal — resuspension/evaporation rate is high and uptake rate is low, an interception sampler (assumed to have a better retention performance compared with 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 when both transport rates are high. Since the results obtained by different sampling procedures are influenced by a range of mass transport processes and may have to be extrapolated beyond the sampled contact volume, many sampling methods are faced with fundamental problems such as the following:

- Interception and retention characteristics of interception techniques differ from real skin or clothing;
- Removal methods, for example, 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, for example, skin washing, are not appropriate for all body parts;
- Extrapolation from small areas sampled, for example, 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 substance of interest.

As indicated, the total mass measured may be a poor surrogate for the uptake, either since the mass of chemical on the skin is not all available for uptake or is spread very unevenly on the skin. There is considerable spatial variation in exposure both between individuals and between anatomical regions, which can currently not be accounted for. Around two-thirds of the total exposure mass is accounted for by the most highly exposed 10% of the body surface area in studies of the Health and Safety Laboratory in the UK (Global Net on ''Consumer Exposure Modelling'': Report of Workshop No. 1 on ''*Dermal transfer and penetration algorithms*'', 2006). This aspect requires further studies and more detailed exposure analyses such as with the Dirichet

Tessellation sampling technique (Wheeler and Warren, 2002). It would further be more relevant to measure the exposure using a sampler, which 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 semipermeable barrier membrane and an impervious backing (Cherrie and Robertson, 1995). Further development of this type of sampler may in the longer term offer a more appropriate measurement method.

Skin absorption

The skin is a living barrier with many functions including the protection of the body from chemical substances to which it is exposed. It is composed of the outermost epidermis, the dermis and an underlying layer of subcutaneous fat. The uppermost layer of the epidermis is the stratum corneum, which is the rate-limiting barrier of the skin. To cross the stratum corneum, a penetrant must first partition between its vehicle and the stratum corneum. There are then three different routes across the stratum corneum (IPCS, 2006): (1) between the cells, through the extracellular lipids (intercellular route), (2) through the cells, partitioning in and out of the cell membranes (transcellular route) and (3) through the shunts of the hair follicles, sweat glands and sebaceous glands (appendageal route). For risk assessment purposes, the chemical adsorbed to the stratum corneum at the end of the experiment is considered as non-bioavailable. The amount of penetrated substance found in the receptor fluid (*in vitro*) or in the blood and carcass (*in vivo*) at the end of the experiment is considered to be systemically available. In addition, the amounts present in the epidermis (minus the stratum corneum) and dermis at that time are often considered to be 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 under *in vivo* conditions (Steiling et al., 2001). The physiology and biochemistry of the human skin can account for much of the variability associated with dermal absorption of substances. Percutaneous absorption varies depending on the site of the body (Wester and Maibach, 1999) and there is also considerable variability at a given site (Feldman and Maibach, 1967) and within and between individuals (Southwell et al., 1984).

The dermal risk assessment approach in the EU is generally to use a No-Observed-Adverse-Effect-Level (NOAEL) from an oral dosing toxicity study in animals. Thus, a route-to-route (oral to dermal) extrapolation is necessary for which data on skin absorption may be used. Quantification of skin absorption is an essential step in reducing the uncertainty of dermal risk assessment. In case no information on skin absorption is available for a substance, it is generally assumed that 100% of the material applied to the skin surface is available systemically. Since for many industrial chemicals, no dermal availability data are available, it should be realized that this extremely conservative default assumption is likely to be applied in many risk assessments under REACH. In various guidance documents (EC, 2003, 2004), the arbitrary value of 10% of the amount contacting the skin is used as a pragmatic basis for risk assessment for compounds with a molecular weight (MW) of more than 500 Da and a logarithm of the octanol-water partition coefficient ($\log P$) of lower than -1 or higher than 4. It is important to emphasize that this is a conservative cut-off value, rather than a quantitative prediction. In the section "Predicting Dermal Absorption with QSAR Techniques" more information on the relationships between absorption and MW and $\log P$ is given.

In Vivo *and* In Vitro *Experimentation*

In practice, quantification of skin absorption is generally based on experimental data, rather than from modeling approaches. In the EU, data from well-performed *in vitro* and/or *in vivo* experiments are considered acceptable.

Animal experiments permit the determination of the penetration of a test substance through the skin into the systemic compartment. The OECD guideline for the conduct of *in vivo* skin absorption studies (TG427; OECD, 2004a) describes a protocol so that the testing and presentation of results may be carried out in the appropriate way. Testing in human volunteers is limited for technical and ethical reasons and their conduct is closely regulated (Declaration of Helsinki, 1964 and updates; World Medical Association, 2004) and the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (ICH, 1996). On the other hand, studies with human volunteers may provide definitive data for the assessment of the absorption of existing chemicals with well-defined toxicological properties (e.g., Hueber-Becker et al., 2006).

Over the last decade, absorption data obtained in *in vitro* studies have been increasingly used in risk assessment of pesticides, biocides, cosmetic ingredients and industrial chemicals in the EU. This technique measures the diffusion of chemicals into and across excised skin to a fluid reservoir. It can be used to measure diffusion (using non-viable skin) or both diffusion and metabolism in the skin (using fresh, metabolically active skin). The OECD guideline for the conduct of *in vitro* skin absorption studies (TG428; OECD, 2004b) presents a harmonized protocol, and various studies have addressed the robustness of *in vitro* methodology (e.g., Van de Sandt et al., 2004; Chilcott et al., 2005). In addition, there is an opinion by the Scientific Committee on Consumer

Products on basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCP, 2006).

There are currently two EU documents containing guidance for the determination of dermal absorption. For new (Dir. 93/67/EEC) and existing (Reg. (EC) No 1488/94) chemicals, as well as for biocides (Dir. 98/8/EC), the Technical Guidance document (TGD) on risk assessment is used as guidance document (EC, 2003), whereas for plant protection products (Dir. 91/414/EEC), guidance is provided by the Guidance Document on Dermal Absorption (EC, 2004). These two documents follow a similar approach regarding the interpretation of dermal absorption studies, although the latter explicitly allows for use of *in vitro* studies as a stand alone methodology to obtain dermal absorption data.

Dermal absorption of chemicals is most often expressed as a percentage of the dose that is in contact with in the skin. Another way to express dermal absorption is by absorption rate or flux (J), which is measured in *in vitro* tests and describes the net rate of transport once equilibrium (steady state) conditions have been reached. The permeability coefficient (K_p) is the flux divided by the total concentration difference across the skin membrane, for measurements made with an infinite dose (see section "Integrating information on exposure and absorption"). Until now, K_p values have not been incorporated in occupational risk assessment. An exception to this rule is the use of K_p values, which are used in the US to estimate the degree of an acute exposure from a large amount/volume of a chemical (incidental splash, contaminated swimming water), which are considered infinite dose scenarios (Walker et al., 1996). Related to this, the US-EPA calls for *in vitro* dermal absorption rate studies (US EPA, 2004a) for a number of chemicals, which are of interest to the Occupational Safety and Health Administration (OSHA) of the Department of Labour.

Predicting Dermal Absorption with Mathematical Modeling

Mathematical modeling 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) and physiological parameters (e.g., blood flow, volume of distribution). Depending on the situation that these mathematical models are describing, they will include additional parameters, such as volume of the vehicle.

Membrane-based mathematical models have been developed for calculating the rate and extent of dermal absorption for a variety of exposure situations. Cleek and Bunge (1993) described one- and two-membrane models for the skin that represent the stratum corneum alone and in combination with the viable epidermis. In these models, it was assumed that the chemical concentration in the vehicle remained constant during the exposure. Bunge and co-workers also proposed strategies for estimating the physicochemical parameters that these models require when the vehicle is water. These models form the basis of the dermal exposure assessment by the US EPA (1992) (2004b). Mathematical models permit using measurements made in one type of experiment to estimate dermal absorption in a different exposure scenario (Kruse et al., 2007). By determining the values of parameters governing the overall skin absorption processes, it is attempted to extrapolate from infinite exposure scenarios to occupational relevant (finite) scenarios.

Predicting Dermal Absorption with QSAR Techniques

Mathematical models are distinctive from (Quantitative) Structure — Activity Relationships ((Q)SAR) models in that QSAR models are used to relate chemical structure to the physicochemical parameters that are important to dermal absorption, that is, permeability coefficients, the measure of a chemical's diffusivity, partition coefficient, the measure of the chemical solubility in the skin layers relative to the vehicle. In general, (Q)SARs attempt to relate the biological activity of a chemical, or series of chemicals, to their physicochemical 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. There have been many attempts to predict permeability coefficients (Cronin, 2005). Approaches to predict skin permeability have ranged from the use of small local data sets, for example for congeneric series, to larger series of compounds. More applicable in terms of industrial chemicals and 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 on data originally collated by Flynn (1990) and extensions of these data. Considering the passage of a chemical through the skin from an aqueous solution, descriptors of hydrophobicity, molecular size and possibly hydrogen bonding (which may describe non-covalent interactions with skin proteins) are of importance for the development of QSARs (Moss et al., 2002). Several studies revealed that skin permeability increased with a linear relationship to hydrophobicity. Particularly when highly hydrophobic substances were included in the database, a parabolic relationship with hydrophobicity was observed (Scheuplein and Blank, 1971). In contrast, the relationship between skin absorption and MW is not clearly established for reasonably sized molecules (de Heer et al., 1999).

Hydrophobicity is well characterized by the logarithm of the octanol — water partition coefficient ($\log K_{ow}$) and molecular size by parameters such as MW. This approach led

Potts and Guy (1992) to develop the following model: $\log K_p = 0.71 \log K_{ow} - 0.0061\,MW - 6.3$ (a computerized version (DERMWIN as part of EPI Suite) can be downloaded from http://www.epa.gov/oppt/exposure/pubs/episuite.htm). 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).

Data from QSARs predicting skin absorption have been used only sparsely in regulatory risk assessment. This is due to the fact that QSARs make predictions of K_p values rather than percent absorption, which is used in regulatory settings and do not take the skin residue into account. All of the published QSARs predicting skin absorption have been developed for compounds dissolved in water, applied to the skin at an infinite dose, which is not realistic for occupational situations. As a result, the nature of substance — vehicle and vehicle — skin interactions are not considered by the existing QSARs. Strategies for making vehicle-to-vehicle adjustments have been proposed (e.g., US EPA, 1992), but more experimental studies supporting these methods are needed. Another important aspect is that skin absorption data can be critical for the overall risk assessment, and that, at present, (Q)SARs do not yet have the required accuracy.

In November 2004, the OECD member countries agreed on the principles for validating (Q)SAR models for their use in regulatory assessment of chemical safety. The agreed OECD principles for the validation of (Q)SAR Models state that to facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information:

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

The principles are intended to be read in conjunction with associated explanatory comments described in http://www.oecd.org/document/23/0,2340,en_2649_34365_33957015_1_1_1_1,00.html. Guidance on how to apply and interprete the principles is also given in this document.

The European Chemicals Bureau made an assessment of the validity of 40 non-proprietary (Q)SARs for *in vitro* skin penetration. In order to establish their scientific validity, the OECD principles for (Q)SAR validation were applied. It appeared that most QSAR publications lack a majority of the needs presented in these principles (dependent on how strict the principles are applied), and that only two QSARs performed reasonably well after external evaluation using a database containing well-defined data from 72 compounds (Bouwman et al., 2006).

As mentioned above, most existing QSARs make predictions of K_p values rather than percent absorption. This raises the question in which way and for which exposure conditions the K_p value will provide a meaningful estimate for dermal absorption. In order to investigate this, K_p values of 16 substances were estimated with the equation of Potts and Guy (1992) based on $\log P$ and MW. Subsequently, the dermal absorption was calculated with the formula $A = K_p C t SA$ (where A = amount absorbed (mg), K_p = permeability constant (cm/h); C = concentration (mg/cm^{-3}), t = exposure time (h) and SA = exposed skin area (cm^2)). The results were subsequently compared with well-defined *in vitro* experimental data from the TNO database and the EDETOX database (http://www.ncl.ac.uk/edetox/index.html). It was shown that the calculations on the basis of modeled K_p values often resulted in a significant overestimation of the measured absorption values and in some cases, in underestimation. The direct use of K_p predictions (based on steady state equations) in risk assessment of chemicals is therefore limited at present to screening of absorption potential. A comparison of the use of relative absorption (% of dose) versus permeability coefficient (K_p) in risk assessment is given in Table 1 by Buist et al. (2005). In addition to percentages or K_p values, dermal flux data may be used (mg/cm^2/h). Although not used at present for risk assessment purposes, this approach allows for variation in time and contact area (CEFIC, 2004), thereby having the potential to provide information which is specific for defined exposure scenarios.

Higher level use of QSAR data on skin absorption is foreseen in the future. Family-specific QSARs equations may be more accurate than QSARs with a large applicability domain, but they all need considerable effort in order to be applicable for quantitative use. An important step may be to investigate the possibility to assign default factors (percent absorption) on the basis of predicted K_p or flux. The advantage of this approach would be that the QSAR predictions would be used to define conservative categories, rather than using the exact outcome of the prediction (for which the existing QSARs lack the required predictivity). This challenge was recently taken up for topically applied cosmetic ingredients by an expert group organized by the European Cosmetic Toiletry and Perfumery Association (Colipa). Experimental data (*in vitro* and *in vivo*) for a broad range of cosmetic ingredients (tested under realistic exposure conditions) indicated that not more than 8% of the applied dose was absorbed dermally in 24 h. It was proposed that conservative default adjustment factors be assigned to four categories of cosmetic ingredients on the basis of calculated maximal fluxes: negligible, 10%, 40% and 80% (Kroes et al., 2007). It was

Table 1. Present use of dermal absorption estimates.

	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	Concentration on the skin surface does not change during exposure
	Amount of substance/cm^2 realistic for worker or consumer	Steady-state flux does not change during exposure period
	Vehicle/formulation realistic for worker or consumer	
Advantages	Experimental conditions can be directly matched to reflect worker exposure conditions	Useful to estimate absorbed dose from different exposure conditions by extrapolation using computational modelling approach
	Can be used for *in vivo* studies	
	Skin depot can be taken into account by adding the percentage dose retained	
Disadvantages	Extrapolation to alternative exposure conditions is difficult	Limited predictivity (at present)
	No QSARs available	

emphasized that this approach involves several worst-case assumptions.

Integrating information on exposure and absorption

There has been surprisingly little interaction between the researchers involved with occupational dermal exposure assessment and those researchers working on dermal permeation. This is a regrettable situation since the two types of information are interlinked and both are necessary for a proper risk assessment of chemicals. Variables such as the area and site of exposure, the duration of exposure, concentration of the chemical and the selected vehicle have a great influence on the absorption of a chemical. There is therefore a clear need for reducing the mismatch between the exposure data obtained in the field or through modelling attempts and experimental or QSAR data on dermal permeation.

Chemical coverage of the skin may be incomplete or exceed the exposed skin surface area, thereby markedly influencing its flux through the skin and the percent absorption. 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. It should be noted that 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 overestimate of the systemically absorbed dose.

Skin absorption of chemicals has been found to be not linear with dose (i.e. mass applied). As the dose per unit area increases, the flux generally increases at a lower degree (when the skin barrier is not affected by the chemical or its vehicle). In a comparative *in vitro* study with 14 chemicals, saturation of the absorption process was frequently observed at higher exposure levels (van Ravenzwaay and Leibold, 2004). In some cases, however, chemicals have been shown to behave quite unexpectedly at different concentrations. The skin absorption of the solvent 2-butoxy ethanol, when applied as an occupationally relevant aqueous mixture, was clearly higher (in absolute terms) than when applied as a neat chemical (Jakasa et al., 2004). The effect of spatial heterogeneity in exposure on absorption has also been examined in experiments with contamined soil (Touraille et al., 2005). These studies show that it is important that the selected doses span the range of doses expected exposure. These should be determined on the basis of quantity per unit area of exposed skin (mg/cm^2). The US EPA (1998) recommended that four, but at least three doses should be used, which should be at log intervals. The OECD test guidelines (427, 428) mention that the test substance preparation should be the same (or a realistic surrogate) as to which humans may be exposed. For pesticides, most often the concentrate formulation and one or two application dilutions are tested.

With respect to the relevance of the dose for skin absorption of chemicals, it should be mentioned that a distinction is made between infinite and finite doses. Infinite dose is defined as the amount of test preparation applied alone or in a vehicle to the skin such that a maximum rate of absorption of the test substance (per unit area of skin) is achieved and maintained (OECD, 2004c). Therefore, the application volume should be large enough that the concentration of the chemical is not depleted.

Under the conditions of a finite dose, the maximum absorption rate may be reached for some of the time, but is not maintained or may not be achieved (OECD, 2004c). This

latter situation is consistent with most occupational and environmental exposures and can be simulated in experimental studies (*in vitro* and *in vivo*). Since existing QSARs have been developed with data obtained from infinite dose experiments, the relevance of their results is therefore limited for quantitative risk assessment purposes.

This was also acknowledged by the European Chemical Industry Council (CEFIC) sponsoring a scientific workshop in 2004 to discuss skin permeation measurement methods (http://www.iom-world.org/news/ppworkshop.php). 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. After this workshop, some progress has been made in this field (Kruse et al., 2007). It should, however, be realized that infinite dose exposure can be assumed in some specific cases. Therefore, calculated K_p values for over 200 organic chemical contaminants in water are used for environmental risk assessment in US Superfund sites (US EPA, 2004b).

It is well known that the nature of the vehicle/formulation in which a substance is exposed to the skin may alter the rate at which it is absorbed, either increasing or decreasing it (Ross and Shah, 2000; Buist et al., 2007). The magnitude of the vehicle effect may be considerable and therefore needs to be taken into account in the risk assessment of substances. For this reason, *in vitro* and *in vivo* testing protocols emphasize the need for exposure conditions which are relevant for the situation of the worker/consumer.

Traditionally, regulatory occupational and environmental exposure assessments have tended to focus on determining systemic exposures resulting from a single exposure scenario, work-shift or day (Van Hemmen and Van der Jagt, 2005). Longitudinal modelling 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 on the probability of long-term overexposure. Additionally, uptake of a chemical following dermal exposure can continue over a number of days so that systemic exposure in a 24-h 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. Proper consideration of the absorption process over multiple days can lead to a more confident prediction of the bioavailabilty 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, by lack of solid information, usually based on high-end exposure percentiles.

Various studies, specifically within the RISKOFDERM project of the fifth EU Framework Programme, have established a large database of dermal exposure levels of chemicals in several occupational use scenarios (Kromhout et al., 2004; RISKOFDERM, 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 into account the protective effect of clothing or the mass of chemical that is likely to be taken up into the body. Since this information is essential for a proper estimation of the internal exposure, there is an urgent need to close this information gap.

Conclusions

Various methodologies exist to measure dermal exposure including surrogate skin and patch methods, removal methods, visualization techniques and biological monitoring. These exposure measurements may provide quantitative or semiquantitative information depending on the study design and quality of the analytical techniques. Although these studies may provide very specific and relevant information, it should be realized that case by case in-depth exposure assessment would be a very expensive process. For this reason, modelling approaches for dermal exposure have been developed. In regulatory practice, a tiered exposure assessment approach is applied in which the first tier is given by a conservative system and subsequent tier(s) providing more refined exposure modelling for scenarios of concern. A first tier might eliminate many substances due to low exposure or toxicity for further investigation. Hence, a generic exposure assessment tool generating scientifically justified and realistic exposure estimates would significantly increase cost-effectiveness of REACH.

Dermal bioavailability data are most often obtained from *in vitro* studies or animal experiments. These methodologies allow for testing under conditions relevant to the occupational setting, with respect to for example, duration of exposure, dose and vehicle/formulation. For a proper assessment of the penetration of a substance through the skin, detailed information on the exposure conditions is necessary. Therefore, there is a need to reduce the uncertainty on dermal exposure estimates due to difficulty in standardization and assumptions that are associated with the method.

Results from non-testing methods such as (Q)SARs have been used only to a very limited extent for regulatory purposes. The permeability coefficient (K_p) of chemicals

present in aqueous solutions can be predicted with some reliability from its physicochemical properties. Caution and additional information, however, are required when using K_p values to estimate risk (i.e. a quantitative determination of internal exposure) following dermal contact. Direct use of K_p values to calculate absorption at finite doses does not appear to produce useful results. The use of K_p or flux values based on QSARs is therefore at present limited for screening purposes (Figure 1). In order to extend the use of non-testing methods for dermal risk assessment of chemical substances, the following research needs are identified:

1. Collection and generation of detailed information on dermal human exposure, fit for the development of improved models for risk assessments. Currently available methods to quantify outcome of dermal exposure provide estimates of mass, loading and concentration have a very limited usefulness. State-of-the-art knowledge on uptake of chemicals by skin needs adequate estimates especially of skin surface exposed, exposure duration, dose (amount and volume per cm^2) and physical state (solid, neat chemical, vehicle/formulation).
2. 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. Since dermal absorption (expressed as a percentage of the available dose) varies with the mass loading of the skin, methodology is required on how to determine the systemic dose from a single value representing the total mass of chemical residing on skin.
3. Methods are needed, which allow extrapolation of (QSAR) data from infinite doses in aqueous solutions to occupationally relevant conditions (finite dose, non-aqueous liquids). It is not realistic to assume that these methods will cover the entire chemical universe. By defining the applicability domain of (Q)SARs with high confidence, the practical use of these data in risk assessment will be stimulated. Most likely, the applicability domain will be limited at first, but could be expanded when new data become available.
4. The definition of conservative categories on the basis of QSAR outcomes, rather than relying on the exact prediction, could be a way forward to gain confidence in the predicted values. More research is needed to determine the more exact range of confidence we can place in QSAR predictions for general use or for read-across using experimental data for a similar molecule.

Exposure scenarios form an essential basis for chemical risk assessment reports under REACH. In case the dermal route of exposure is predominant, information on exposure and dermal bioavailability is necessary for a proper risk assessment. Despite the fact that dermal availability is not mentioned as a formal information requirement in the annexes in the REACH text, it is therefore anticipated that such information will be needed for the risk assessment for many substances.

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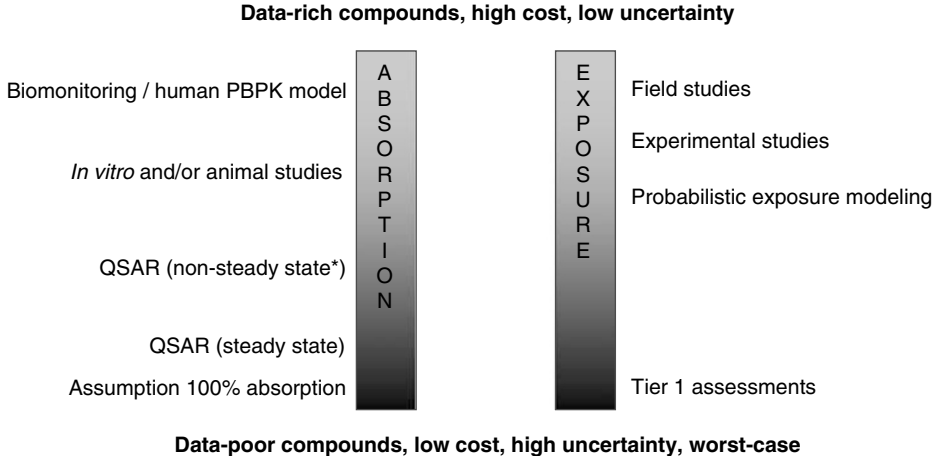

Figure 1. Tiered approaches for data generation on skin absorption and exposure. *Non-steady state equations are not available at present.

(Global Net on "Consumer Exposure Modelling": Report of Workshop No. 1 on "*Dermal transfer and penetration algorithms*", 2006): ten Berge, Wil, Santoxar, Westervoort, The Netherlands; Bunge, Annette L., Colorado School of Mines, Golden, CO, USA; Corish, John, University of Dublin, Dublin, Ireland; Cronin, Mark, Liverpool John Moores University, Liverpool, England; Diembeck, Walter, Beiersdorf A.G., Hamburg, Germany; de Heer, Cees, TNO Quality of Life, Zeist, The Netherlands (now: RIVM, Bilthoven, The Netherlands); van der Jagt, Katinka E., EMEA, London, England (now: DG Enterprise EU, Brussels, Belgium); Kephalopoulos, Stelios, JRC/IHCP/PCE, Ispra, Italy; Kissel, John, University of Washington, Seattle, WA, USA; McCready, David, The Dow Chemical Company, South Charleston, West Virginia, USA; Papamelitiou, Demosthenos, JRC, IHCP, PCE, Ispra, Italy; Patlewiecz, Grace, JRC/IHCP/ECB, Ispra, Italy; Perkins, John, Dow Agroscience Ltd., Milton Park Abingdon, England; Ross, John, Infoscientific, Carmichael, CA, USA; Steiling, Winfried, 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 and Worth, Andrew, JRC/IHCP/ECB, Ispra, Italy.

References

Bouwman T., Cronin M.T.D., Bessems J.G.M., and Van de Sandt J.J.M. Evaluation of published QSARs for percutaneous absorption. *Toxicol Lett* (2006): 164: S322.

Brouwer D.H., Aitken R.J., Oppl R., and Cherrie J.W. Concepts of skin protection: considerations for the evaluation and terminology of the performance of skin protective equipment. *J Occup Environ Hyg* (2005): 2: 425–434.

Buist H.E., de Heer C., Bessems J.G.M., Bouwman T., Chou S., Pohl H.R., and Van de Sandt J.J.M. Dermal absorption in risk assessment: the use of relative absorption versus permeation coefficient (K_p). *Occupational and Environmental Exposures of Skin to Chemicals — 2005* (2005). Abstract for poster 25 http://www.cdc.gov/niosh/topics/skin/OEESC2/AbPost025Buist.html.

Buist H.E., de Heer C., van Burgsteden J.A., and van de Sandt J.J.M. Dermatokinetics of didecyldimethylammonium chloride and the influence of some commercial biocidal formulations on its dermal absorption *in vitro*. *Regul Toxicol Pharmacol* (2007) (E-pub ahead of print).

CEFIC. CEFIC Workshop on methods to determine dermal permeation for human risk assessment. Research Report TM/04/07 December 2004 (2004), 1–86. http://www.iom-world.org/news/ppworkshop.php.

Cherrie J.W., and Robertson A. Biologically relevant assessment of dermal exposure. *Ann Occup Hyg* (1995): 39: 387–392.

Chilcott R.P., Barai N., Beezer A.E., Brain S.I., Brown M.B., Bunge A.L., Burgess S.E., Cross S., Dalton C.H., Dias M., Farinha A., Finnin B.C., Gallagher S.J., Green D.M., Gunt H., Gwyther R.L., Heard C.M., Jarvis C.A., Kamiyama F., Kasting G.B., Ley E.E., Lim S.T., McNaughton G.S., Morris A., Nazemi M.H., Pellett M.A., DU Plessis J., Quan Y.S., Raghavan S.L., Roberts M., Romonchuk W., Roper C.S., Schenk D., Simonsen L., Simpson A., Traversa B.D., Trottet L., Watkinson A., Wilkinson S.C., Williams F.M., Yamamoto A., and Hadgraft J. Inter- and intralaboratory variation of *in vitro* diffusion cell measurements: an international multicenter study using quasi-standardized methods and materials. *J Pharm Sci* (2005): 94: 632–638.

Cleek R.L., and Bunge A.L. A new method for estimating dermal absorption from chemical exposure. 1. general approach. *Pharm Res* (1993): 10(4): 497–506.

Cronin M.T.D. 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, 2005.

De Heer C., Wilschut A., Stevenson H., and Hakkert B.C., Guidance document on the estimation of dermal absorption according to a tiered approach: an update. Zeist, Netherlands, TNO report V98.1237 (1999), 1–42. To be obtained through J.J.M. van de Sandt (han.vandesandt@tno.nl).

EC. Technical guidance document (TGD) on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances. *European Commission, Part, 1* (2003), 1–303.

EC. Guidance document on dermal absorption. *European Commission, Sanco/222/2000 rev. 7* (2004), 1–15.

Feldman R.J., and Maibach H.I. Regional variation in percutaneous penetration of ^{14}C cortisol in man. *J Invest Dermatol* (1967): 48: 181–183.

Fitzpatrick D., Corish J., and Hayes B. Modelling skin permeability for risk assessment — the future. *Chemosphere* (2004): 55: 1309–1314.

Flynn G.L. Physicochemical determinants of skin absorption. In: Gerrity T.R., Henry C.J. (Eds.). *Principles of Route-to-Route Extrapolation for Risk Assessment*. Elsevier: New York, 1990, pp 93–127.

Global Net on "Consumer Exposure Modelling". Report of Workshop No. 1 on "Dermal transfer and penetration algorithms". (2006). (Stylianos Kephalopoulos, Joop J. van Hemmen, Katinka van der Jagt). EUR 22521 EN/1 — DG Joint Research Centre, Institute for Health and Consumer Protection, Luxembourg: Office for Official Publications of the European Communities.

Hueber-Becker F., Nohynek G.J., Dufour E.K., Meuling W.J.A., de Bie AThHJ , Toutain H., and Bolt H.M. Occupational exposure of hairdressers to [(14)C]-para-phenylenediamine-containing oxidative hair dyes: a mass balance study. *Food Chem Toxicol* (2006) (E-pub ahead of print).

ICH (International conference on harmonization). Guideline for good clinical practise. *ICH Harmonised Tripartite Guideline* (1996), 1–57. http://www.ich.org.

IPCS. International Programme on Chemical Safety, Environmental Health Criteria 235: dermal absorption. *United Nations Environment Programme, World Health Organisation* (2006), WHO Press: Geneva, Switzerland.

Jakasa Y., Mohammadi N., Kruse J., and Kezic S. Percutaneous absorption of neat and aqueous solutions of 2-butoxyethanol in volunteers. *Int Arch Occup Environ Health* (2004): 77: 79–84.

Kroes R., Renwick A.G., Feron V., Galli C.L., Gibney M., Greim H., Guy R.H., Lhuguenot J.C., and Van de Sandt J. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients, (2007) (submitted).

Kromhout H., Fransman W., Vermeulen R., Roff M., and Van Hemmen J.J. Variability of task-based dermal exposure measurements from a variety of workplaces. *Ann Occup Hyg* (2004): 48: 187–196.

Kruse J., Golden D., Wilkinson S., Williams F., Kezic S., and Corish J. Analysis, interpretation, and extrapolation of dermal permeation data using diffusion-based mathematical models. *Pharm Sci* (2007): 96: 682–703.

Moss G.P., Dearden J.C., Patel H., and Cronin M.D. Quantitative structure-permeability relationships (QSPRs) for percutaneous absorption. *Toxicol In Vitro* (2002): 16: 299–317.

OECD. OECD guideline for the testing of chemicals. *Skin Absorption: In Vivo Method* (2004a): 427. Adopted: 13 April 2004, pp 1–8, OECD publishing, Paris.

OECD. OECD guideline for the testing of chemicals. *Skin Absorption: In Vitro Method* (2004b): 428. Adopted 13 April 2004, pp 1–8, OECD publishing, Paris.

OECD. Guidance document for the conduct of skin absorption studies. *OECD Series on Testing and Assessment* (2004c) Number 28 ENV/JM/MONO (2004), pp 21–31, OECD publishing, Paris.

Potts R.O., and Guy R.H. Predicting skin permeability. *Pharm Res* (1992): 9: 663–669.

RISKOFDERM. Special Issue. *Ann Occup Hyg* (2004): 48: 183–297.

Ross J.S., and Shah J.C. Reduction in skin permeation of *N,N*-diethyl-*m*-toluamide (DEET) by altering the skin/vehicle partition coefficient. *J Control Release* (2000): 67: 211–221.

SCCP (Scientific Committee on Consumer Products). Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients. Document SCCP/0790/06 Adopted by the SCCP on March 28 (2006).

Scheuplein R.J., and Blank I.H. Permeability of the skin. *Physiol Rev* (1971): 51: 702–747.

Southwell J.D., Barry B.W., and Woodford R. Variations in permeability of human skin within and between specimens. *Int J Pharm* (1984): 18: 299–309.

Steiling W., Kreutz J., and Hofer H. Percutaneous penetration/dermal absorption of hair dyes *in vitro*. *Toxicology In Vitro* (2001): 15: 565–570.

Touraille G.D., McCarley K.D., Bunge A.L., Marty J.P., and Guy R.H. Percutaneous absorption of 4-cyanophenol from freshly contaminated soil *in vitro*: effects of soil loading and contamination concentration. *Environ Sci Technol* (2005): 39: 3723–3731.

US EPA. Dermal exposure assessment: principles and applications. EPA/600/8-91/011B (1992), 1–389. http://www.epa.gov/nceawww1/pdfs/derexp.pdf.

US EPA. Health effects test guidelines. US Environmental Protection Agency, OPPTS 870.7600. Dermal penetration (1998), 1–12. http://www.epa.gov/opptsfrs/OPPTSHarmonized/870HealthEffectsTestGuidelines/Series/870-7600.pdf.

US EPA. *In vitro* dermal absorption rate testing of certain chemicals of interest to the occupational safety and health administration; Final rule. *Fed Regist* (2004a): 69(80): 22402–22441.

US EPA. Risk assessment guidance for superfund volume I: human health evaluation manual (part E, supplemental guidance for dermal risk assessment). EPA540/R/99/005 (2004b), 1–156. http://www.epa.gov/oswer/riskassessment/ragse/pdf/20041101pa rte.pdf.

Van de Sandt J.J.M., van Burgsteden J.A., Carmichael P.L., Dick I., Kenyon S., Korinth G., Larese F., Limasset J.C., Maas W.J.M., Montomoli L., Nielsen J.B., Payan J.-P., Robinson E., Sartorelli P., Schaller K.H., Wilkinson S.C., and Williams F.M. *In vitro* predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. *Regul Toxicol Pharmacol* (2004): 39: 271–281.

Van Hemmen J.J., and Brouwer D.H. Assessment of dermal exposure to chemicals. *Sci Total Environm* (1995): 168: 131–141.

Van Hemmen J.J., and Van der Jagt K.E. Generic Operator exposure databases. In: Franklin C.A., Worgan J.P. (Eds.). *Occupational and Residential Exposure Assessment for Pesticides*. Wiley and Sons: UK, 2005.

Van Ravenzwaay B., and Leibold E. The significance of *in vitro* rat skin absorption studies to human risk assessment. *Toxicology In Vitro* (2004): 18: 219–225.

Walker J.D., Whittaker C., and McDougal J.N. Role of the TSCA Interagency Testing Committee in Meeting the US Government's Data Needs: Designating chemicals for percutaneous absorption testing. In: Marzulli F.N., Maibach H.I. (Eds.). *Dermatotoxicology*, 5th edn Hemisphere Publishing Corporation: Washington, DC, (1996), 371–381.

Wester R.C., and Maibach H.I. Regional variation in percutaneous absorption. In: Bronaugh R.L., Maibach H.I. (Eds.). *Percutaneous Absorption: Drugs—Cosmetics — Mechanism — Methodology*. (3rd ed., rev. and expanded). [Drugs and pharmaceutical science Vol. 97] Marcel Dekker Inc: New York, (1999), pp 107–116.

Wheeler J.P., and Warren N.D. A Dirichlet Tesselation-based sampling scheme for measuring whole-body exposure. *Ann Occup Hyg* (2002): 46: 209–217.

WMO (World Medical Association). Declaration of Helsinki (Ethical principles for medical research involving human subjects) (2004), 1–5. http://www.wma.net.

Zartarian V., Bahador T., and McKone T. Adoption of an official ISEA glossary. *J Expo Anal Environ Epidemiol* (2005): 15: 1–15.