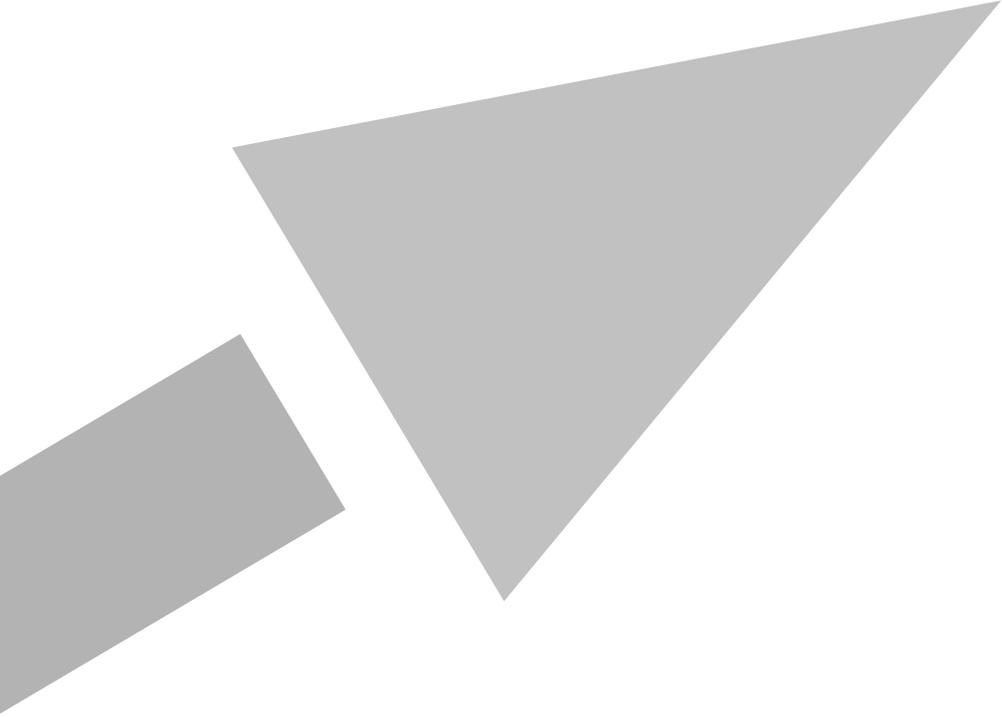


***Evaluation of Systemic
Health Effects Following
Dermal Exposure to Chemicals***

Technical Report No. 119



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Evaluation of Systemic Health Effects Following Dermal Exposure to Chemicals

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SUMMARY

Risk assessment of chemicals, including the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation, also cover assessments of dermal risk arising from exposure to chemicals. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has produced this guidance consisting of a tiered approach for the risk assessment of dermal exposure to a chemical.

The process developed by the Task Force consists of three linked decision trees, i.e.:

- Derivation of a health-based reference value.
- Initial risk characterisation.
- Refined risk characterisation.

It is designed to use existing exposure and toxicology data and identify data gaps or data inadequacies to be addressed to complete a risk assessment.

Each component of the decision trees is supported by explanation in the text and, in relevant cases, by an appendix providing additional detailed background information. This allows users with varying amounts of experience in risk assessment to access the guidance at different levels.

The decision trees are structured to allow early identification of potential data gaps and to provide guidance on a variety of options such as acquiring basic toxicology data, the use of QSAR/computer-based predictions or the generation of dermal absorption data. Options for the refinement of exposure assessment range from revising parameters in the models to the conduct of bio-monitoring studies in exposed individuals. The process of a tiered approach can be ended at any stage depending on the outcome of the risk assessment. All non-animal refinement options should be considered to allow the potential demonstration of an adequate margin of safety in risk assessment before opting to perform new toxicology/ADME tests in animals.

Several case studies are presented to illustrate how the decision process can be utilised.

1. INTRODUCTION

In the chemical industry, developing approaches to assess the health risks associated with dermal exposure and skin penetration of chemicals has typically received less emphasis than other exposure scenarios, especially inhalation. With improved controls of inhalation exposures, the relative contribution of dermal exposure and subsequent skin penetration to potential health risks has become higher.

There are many occupational circumstances through which skin contact may arise and examples of these are contained in the Technical Guidance Documents (TGD) for the implementation of REACH; guidance on information requirements and chemical safety assessment (in this document referred to as REACH TGD); part D deals with exposure scenarios. Some situations are more obvious than others. For example, a worker may come into direct prolonged contact with chemicals in the form of solids or liquids without any form of protection such as gloves, while the use of personal protective equipment reduces dermal exposure. There may still be some skin exposure as the degree of protection afforded by protective equipment can be variable depending on the specification given to the equipment, e.g. the barrier efficiency of gloves is dependent on the chemical, glove material and the resultant breakthrough time.

Alternatively, there may be situations where a chemical is aerosolised and the chemical may end up on the skin (as well as being inhaled) and this situation could lead to concomitant exposure via the dermal and inhalation routes. Further, some chemicals that come into contact with skin can be quickly volatilised, thus altering the degree and duration of exposure.

To address the evaluation of systemic health effects following dermal exposure, ECETOC convened a Task Force of experts to address the following terms of reference.

Accounting for recent scientific advances, develop a tiered, practical strategy for the assessment and evaluation of dermal risks from chemicals that are based (as far as practicable) on an assessment of the current scientific understandings of the following critical areas:

- Dermal exposure and dermal absorption of substances.
- Effects arising from dermal exposure with a focus on systemic effects.
- Approaches for the acquisition of relevant data (physicochemical, exposure, toxicological and clinical data).
- The manner in which dermal exposure and risk is assessed under relevant regulations, for example REACH ('exposure scenarios').
- Illustration of the utility of the proposals via the use of case studies.

The aim of this document is to provide simple and effective guidance on the procedures to follow when undertaking an assessment of human health risks from dermal exposure to chemicals in **occupational settings** and in relation to possible **systemic effects**.

While the guidance is intended to support the implementation of legislation such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in the EU (EU, 2006) or the US HPV (high production volume) programme (www.epa.gov/hpv). Parts may also be relevant to industry in a broader context. The guidance is focussed on the occupational setting, but still general in the sense that it is also useful for down-stream users, except where specific guidance is available, e.g. for agrochemicals, biocides and cosmetic products. Further, this guidance focuses primarily on the procedures to follow in assessing systemic effects. Specific areas of local toxicity, e.g. corrosion, irritation and sensitisation, are addressed in other ECETOC guidance (ECETOC 1990a; 1990b; 1995; 1999a; 1999b; 2000; 2002; 2003; 2008).

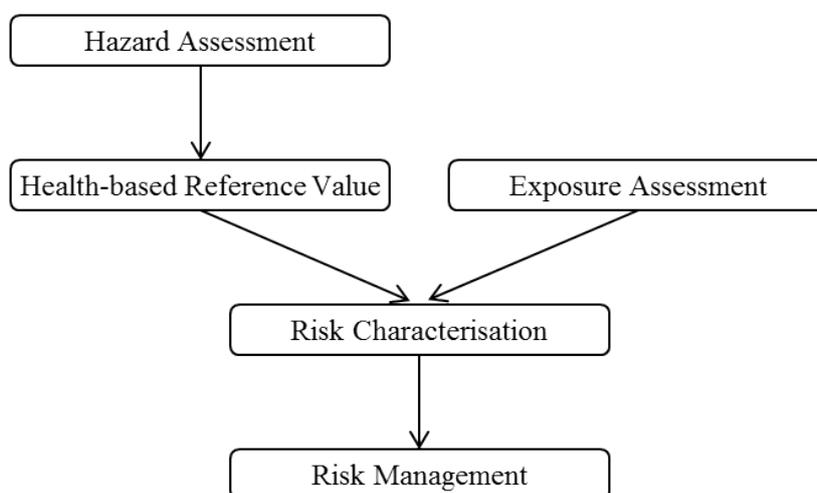
2. DEFINITIONS

This ECETOC report requires definitions in the area of dermal absorption as there are slight divergences in the use of specific phrases/terminology within various official documents and in the published literature. The definitions as proposed by the International Programme on Chemical Safety (IPCS, 2006) are used in this report.

The **percutaneous/dermal absorption** process is a global term which describes the passage of compounds across the skin. This process can be divided into three steps:

- **Penetration**, which is the entry of a substance into a particular layer or structure such as the entrance of a compound into the *stratum corneum*.
- **Permeation**, which is the penetration through one layer into another, which is both functionally and structurally different from the first layer.
- **Resorption**, which is the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

The general risk assessment process is shown in the scheme pictured below. The derivation of a health-based reference value is described in Section 3.1; risk characterisation schemes are outlined in Sections 3.2 and 3.3.



3. METHODOLOGY

This chapter provides stepwise guidance on assessing dermal risk from chemicals with additional clarification and links in the associated appendices.

A tiered approach is described which starts with the evaluation of existing toxicity data (not just relating to the dermal route but to all data for the substance). In addition, data gaps or data inadequacies can be defined to be addressed before conducting a refined assessment based either on modelled data or based on experimental data. The latter usually requires generation of dermal absorption data or a study to determine the systemic dose resulting from a specific exposure scenario, and/or bio-monitoring.

This approach has been laid out in terms of three linked decision trees:

- Derivation of a health-based reference value.
- Initial risk characterisation.
- Refined risk characterisation.

The decision trees should be considered in conjunction with the relevant regulatory requirements. The process can be terminated at any stage based upon an acceptable risk characterisation ratio. Case studies have been included as examples of how the guidance can be utilised (Chapter 4).

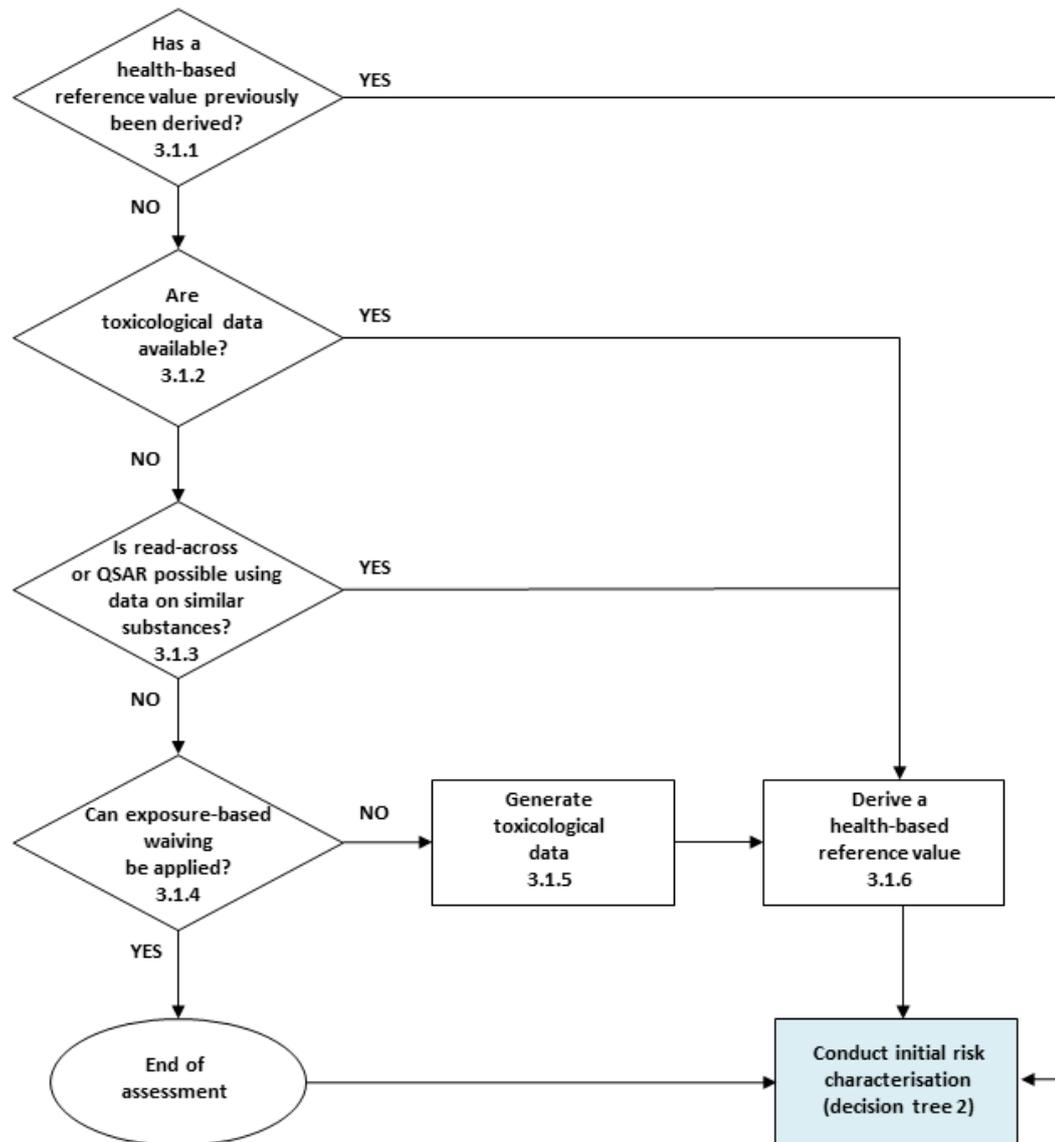
3.1 *Derivation of a health-based reference value*

Deriving a dermal health-based reference value is required in cases where exposure to a chemical can occur via the dermal route. In cases of proven zero exposure, it is not necessary to derive a health-based reference value.

When dermal exposure occurs, a chemical can pass through the skin and into systemic circulation. This entry into the body can potentially result in tissue or systemic damage remote to the site of skin contact.

The process for identifying whether a health-based reference value already exists or can be generated is outlined in Figure 1.

Figure 1: Derivation of a health-based reference value



Before using this scheme, there are some underlying principles that need to be taken into consideration.

Health-based reference values as set by authoritative bodies may have different degrees of uncertainty. The basis for the incorporation of any uncertainty factors should be transparent. Health-based reference values for genotoxic carcinogens are usually based on a specified risk level. Health-based reference values are usually derived from toxicology studies, i.e. the effect or no-effect concentrations at certain doses of exposure. But in some instances a ‘policy’ decision

may have been taken resulting in the setting of a pragmatic or precautionary guidance value. The basis of the value should be known before using it in a risk assessment context.

An appropriate health-based reference value derived from toxicological effects data should be chosen such that a quantitative comparison can be made with the estimated skin exposure (external dose) or the body burden (internal systemic dose), meaning that the units of exposure need to be comparable with the reference value.

It may be necessary to consider the impact on specific groups and sub-populations (e.g. workers, consumers and humans liable to indirect exposure via the environment) and possibly for certain vulnerable sub-populations (e.g. children, pregnant women) and for different routes of exposure. In these cases, the length of potential exposure (acute, short-term or long-term) may be relevant; body weight should be adjusted as required.

For dermal risk assessments specifically, a dermal health-based reference value can, if necessary, be derived from other routes of exposure, such as oral or inhalation or, if no other information is available, from intravenous or intra-peritoneal administration studies. If more than one route of exposure is likely to occur concomitantly, (where possible) separate health-based reference values may need to be established for each route of exposure and the exposure from all routes combined. In the instance where a NO(A)EL can be taken from existing studies or derived using a QSAR model, it may need to be corrected for the relevant route of dosing/exposure.

Guidance for the generation of health-based reference values can be found from various sources. A report from the Institute of Environment and Health in the UK provides some guidance into factors that can be applied with respect to route-to-route extrapolations of health-based reference values (IEH, 2006). The REACH TGD, Chapter R.8, provides 'default' assessment factors accounting for differences in route-to-route extrapolation, inter- and intra-species differences, etc. ECETOC has published further guidance on how to apply assessment factors to derive a DNEL (ECETOC, 2010).

If a study has been performed via the dermal route and a NO(A)EL, expressed as delivered topical dose, has been derived from this study, the value does not need to be corrected for route of exposure. However, such a study will be specific for the dermal route, and is only relevant in this context, particularly if no effects have been seen as a result of negligible systemic exposure. Also, the impact of vehicle or formulation/use of the neat chemical needs to be considered and appropriate corrections applied.

In the absence of compound-specific data, Figure 1 provides a process of systematically reviewing the available data sources to generate a health-based reference value. Some segments of the chemical industry (e.g. the agrochemical industry) are 'data-rich' as a result of specific

regulatory requirements. Other segments of the chemical industry, which cover potentially >10000 chemicals, may have more limited data sets. The scheme outlined in Figure 1 covers these extremes and provides for data from various sources to be considered when deriving a health-based reference value. It is envisaged that data from multiple sources can be used, and the following sections describe them in more detail. These sources are not necessarily to be considered in sequence but can also be taken into account in parallel.

3.1.1 Has a health-based reference value previously been derived?

The starting point is to inquire whether the substance in question has already been evaluated by an authoritative body for human health hazards and thus a health-based reference value has already been derived. The basis and units for this value should be understood. Care should be taken in noting which data were used as the pivotal/critical study input to the derivation and what uncertainty/assessment factors are incorporated into the final derivation of a health-based reference value.

For a limited number of substances only, usually those with a high production volume, reliable assessments, including a review of all available toxicology data, are available e.g. in reports from the US National Toxicology Program (NTP), the WHO International Agency for Research on Cancer (IARC), the (former) European Chemicals Bureau (ECB), REACH chemical safety reports (CSR) or from the OECD screening information data sets (SIDS). Reports can be found on the respective websites.

Compilations of hazard data are also available from several publicly accessible databases. The US Environmental Protection Agency's Integrated Risk Information System (IRIS) contains well-researched data intended for use in risk assessments, decision-making and regulatory activities. The information in IRIS is intended for individuals without extensive training in toxicology, but with some knowledge of health sciences. However, this database focuses on toxicology studies from oral and inhalation dosing and contains few data from dermal dose studies. Where data are available via the dermal route of exposure, the vehicle/formulation used for the study should be noted and an interpretation made as to the relevance of the vehicle in a risk assessment context, since vehicle effects can significantly impact the extent of skin absorption of the test substance.

The European Chemical Substances Information System (ESIS) contains all substances registered under EU chemicals and biocides regulations; the amount and quality of the information present in ESIS varies from substance to substance. For substances that are listed on EINECS (European Inventory of Existing Commercial Chemical Substances, according to the Dangerous Substances Directive; EEC, 1967; EC, 1993) or ELINCS (European List of Notified Chemical Substances, according to the Dangerous Substances Directive), the official classification, as now laid down in

Annex 1 of the REACH legislation (EU, 2006), can easily be obtained from ESIS (see website of JRC: <http://esis.jrc.ec.europa.eu/>). For substances on ELINCS, this information is likely to be correct and up-to-date. For substances on EINECS, producers and/or importers may have self-classified the substance based on the outcome of their hazard assessment of the substance.

In terms of classification, an initial assessment should be made as to whether the substance under consideration is considered dangerous by any EU toxicological criterion. Data to answer this question may be found in the IUCLID (International Uniform Chemical Identification Database) files stored in ESIS, in IRIS or in other toxicological information data-bases, such as RTECS (Registry of Toxic Effects of Chemical Substances) which contains basic toxicological information on a large number of chemicals, or ATSDR (Agency for Toxic Substances and Disease Registry) which gives detailed toxicological information on a limited number of substances.

To harmonise EU legislation with the Globally Harmonised System for Classification and Labelling (GHS), a new Regulation on Classification, Labelling and Packaging (CLP) was introduced in 2008 (EU, 2008). New classifications and labelling for a large number of substances were published in this legislation, and it gives detailed rules for self-classification of substances under GHS. If a data set for a substance is available and it is not classified based on the rules laid down in the CLP, it is to be considered non-hazardous for human health.

3.1.2 Are toxicological data available?

If the substance has not been evaluated or classified previously, then examination of toxicology data available in the public domain is required. A comprehensive search of all potential sources of toxicology study data should be performed. Typically, data may be available from the following toxicological studies with relevance to evaluating systemic toxicity:

- Acute toxicity;
- repeat-dose (target organ) toxicity;
- reproductive and developmental toxicity;
- mutagenicity;
- carcinogenicity.

When searching for studies, it is important to look for data from any route of administration, including oral and inhalation routes as data from these studies can also be used to derive a health-based reference value for dermal exposure-based risk characterisation, with the application of route-to-route specific assessment factors. Data from other routes (e.g. intraperitoneal, intravenous) may also provide information that is helpful for classifying hazards, or for characterising the kinetic handling of a chemical, but are generally less suitable for use in deriving a health-based reference value.

Even though the risk assessment to be performed is on systemic effects, a search for studies on local skin effects via the dermal route should be performed, as damage to the skin can influence the degree of systemic exposure and consideration of this may contribute to the final risk assessment.

If suitable studies are found, the data should be evaluated by a competent toxicologist, with reference to guidance available on the conduct of specific study types. There are numerous publications covering comprehensive guidance on each type of toxicological study, the most recent one being the REACH Guidance on Information Requirements and Chemical Safety Assessment (REACH TGD).

Evaluation of the scientific rigour of the data is crucial to define the pivotal study used in deriving a health-based reference value. A coding system has been developed by Klimisch to score animal studies for their reliability and quality (Klimisch et al, 1997) and to assist in choosing the best study as a valid starting point (e.g. in defining a NOEL or a benchmark dose) and then going on to derive a health-based reference value.

Systemic effects

Acute toxicity studies that generate an LD₅₀ value are of limited value to risk assessment, but can give a crude indication of a substance's toxic potential. Repeat-dose toxicity studies designed to assess chronic systemic effects may indicate no toxicity at any given dose or that specific types of toxicity could arise at a certain dose. The types of effect will be described in the study reports, but could relate to any target organ pathology or any systemic effects (such as reproductive, teratogenic, tumorigenic, endocrine, immunologic or neuronal). Various methods can be used to establish quantitative toxicological data for different effects, and the data are usually described in the form of either:

- LD₅₀, LC₅₀ or MTD – from acute studies;
- no observable (adverse) effect level (NO(A)EL) – from sub-chronic/chronic studies;
- lowest observable (adverse) effect level (LO(A)EL) – from sub-chronic/chronic studies;
- benchmark dose (BMD) – from sub-chronic/chronic studies.

These levels are specific to the different types of endpoints described in a study. It is important to establish either a NO(A)EL or a BMD for the most sensitive systemic effect of concern, and use this in the overall risk assessment. If studies indicate that there are systemic effects, it is to be considered whether all systemic effects (e.g. those described in the REACH TGD) have been covered in the study or whether there are data gaps.

The impact of local effects

When a chemical comes in contact with the skin, irritation, sensitisation or inflammation at the site of contact can result in a local effect. If the chemical or preparation is classified as irritant, this should be taken into consideration when undertaking read-across for absorption.

This is acknowledged in the assessment factors that are applied in the generation of a DNEL (REACH TGD, Chapter R.8; ECETOC, 2010).

In extreme cases, where substances are corrosive and directly damage the skin, this can have a significant impact on dermal absorption, and this should be factored into the overall risk assessment.

Guidance on defining and determining corrosiveness is found in ECETOC Monograph 15 (ECETOC, 1990b). Substances or preparations are considered corrosive if they have strong acidic or alkaline reactions indicated by a pH of 2 or less or 11.5 or greater. In addition, (organic) peroxides and hydroperoxides are considered corrosive unless there is clear evidence to the contrary. Testing can be carried out also by use of an appropriate validated *in vitro* test (OECD 430, 431, 2004a,b).

Mixtures should be considered corrosive if they contain more than 1% or 3% of impurities or additives that are classified as Cat. 1 or Cat. 2 corrosives, respectively, and additivity does not apply (e.g. strong acids and bases). For most substances classified as Cat. 1 corrosives, additivity will apply and mixtures containing such substances will be considered corrosive if the concentration is $\geq 5\%$. If the substance or mixture is considered corrosive, in most circumstances a dermal risk assessment is not warranted as any skin exposure in the workplace should be avoided. Upon dilution of the corrosive material, it may cause local effects to the skin, and it should be considered whether an appropriate uncertainty factor could be applied to the derivation of a health-based reference value in the risk characterisation.

3.1.3 Is read-across or QSAR possible using data on similar substances?

In situations where there are no toxicology endpoint data available on the exact substance of interest, it may be possible in some circumstances to predict the hazards and health-based reference value of the substance from data available on a related substance or category of substances that can be regarded as chemically and/or toxicologically 'similar'. This principle is termed 'read-across' and in its simplest form, the toxicology data for substance X can be transferred directly or used to predict the toxicological properties of the similar substance Y, or to infer the toxicological properties of a group of analogues. The ECHA 'Practical Guide 6: How to report read-across and categories' (ECHA, 2009a) states in:

Section 2.2: “Substances whose physicochemical and/or toxicological and/or ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity, may be considered as a group, or ‘category’ of substances. These similarities may be due to a number of factors:

- Common functional group*
- Common precursor or breakdown products*
- Constant pattern in changing potency*
- Common constituents or chemical class.”*

Section 2.4: “It is important that the chemical structures and purity profiles of all category members are well defined to establish the category hypothesis, since differences in impurities or stereochemistry can affect the activity and chemical properties. Additionally, the chemical structures of all constituents of the registered substance (including impurities, if relevant) need to be well identified.”

When structural properties of a family of similar chemicals exhibit a qualitative relationship to toxicological hazard properties, a Structure Activity Relationship (SAR) may be developed to support read-across. If a measure of the quantitative dose-response nature of the toxicological effect can be related to the chemical properties of a family of similar chemicals using mathematical modelling, then a Quantitative Structure Activity Relationship (QSAR) can be developed. However, using modelled data in a quantitative way (if at all possible for hazard identification and characterisation) might be associated with a high level of uncertainty in the derivation of a health-based reference value. ECHA also issued a ‘Practical Guide 5: How to report (Q)SARs’ (ECHA, 2009b).

Within REACH, special consideration should be given to the use of alternative data (e.g. *in vitro* data, QSAR, read-across or chemical categories) in preference above performing additional animal studies, and if considered justified to fill a data gap. Chapter R.6 of the REACH TGD provides guidance on ‘QSARs and grouping of chemicals’.

Some guidance on the concept of chemical categorisation is also provided in relation to the US High Production Volume (HPV) Challenge program which is published in the OECD ‘Manual for investigation of HPV chemicals’ (OECD, 2005).

Significantly more scientific guidance is needed on defining how chemicals can be categorised as ‘similar’ within a class to allow read-across to be used for the purposes of regulatory classification, labelling or risk assessments. Rila et al (2006) recommend that definitions be developed for ‘common functional groups’ to define categories for read-across. However, this is also not trivial as substances may possess multiple functional groups.

Assessing the similarities of substances is complex and requires case by case evaluations of supporting data to define what similar means in each evaluation. It should be noted that substances which appear chemically similar in terms of structure can have different toxicological effects and therefore read-across should be done with care and with good supporting evidence. The criteria for similarity between substances need be confirmed with scientific rigour by careful analysis of all chemical and toxicological properties. Wu et al (2010) have provided a framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogues for SAR-based toxicological assessments.

New computational tools are being developed to support chemical categorisation approaches and read-across, e.g. Toxmatch as illustrated in the report by Gallegos Saliner et al (2005). Tools to group chemicals can be found in the OECD QSAR Toolbox, which is freely available at <http://www.qsartoolbox.org/>. Commercial software is also available to enable read-across.

In some instances, there may be enough toxicological data on a family of similar chemicals to build a QSAR model to predict an effect, though these instances are expected to be very rare for systemic toxicity (Appendix A).

Even if QSAR cannot be used with sufficient confidence to predict a health-based reference value *per se*, the technique of read-across could be used qualitatively to classify the likelihood of a new chemical acting as a particular type of toxin and guide a decision on what is expected to be the most sensitive endpoint test to perform and thus lead to ‘intelligent testing strategies’.

The REACH TGD indicates that read-across and chemical category approaches should be considered together with both exposure (in this case dermal) and the perceived risks, i.e. if exposures are low and the hazard potential (from read-across/QSAR predictions) is expected to be of low concern, this information together with using the weight of evidence may provide enough combined information for a risk assessment outcome to be reached. When no information can be gained from read-across/QSAR or a health-based reference value cannot be derived from these approaches, an alternative approach to risk assessment in low exposure scenarios would be to consider exposure-based waiving alone.

3.1.4 Can exposure-based waiving be applied?

In cases where toxicological data neither exist for a particular substance nor for substances similar to the substance under consideration, pragmatic approaches, such as ‘exposure-based waiving’, may be considered first before embarking on costly and animal-intensive *in vivo* study data generation. When human and – for the purpose of this document – dermal exposure can be excluded, the search for a health-based reference value becomes superfluous as the risk assessment will obviously arrive

at ‘no significant risk’. In cases where exposure is deemed to be ‘low’, the conclusion of ‘no significant risk’ can only be given if there is appropriate supporting evidence.

In the case where no classification for human health endpoints has been assigned to a chemical, the potential for adverse health effects in humans is deemed to be low. An assessment of the magnitude of exposure could lead to exposure-based waiving, if it is sufficiently low.

Under REACH, there are justifications for such waiving that may be used including the absence of or insignificant/unlikely exposure. Examples for the latter are strictly-controlled conditions applied to the whole life-cycle of a substance and substances that are incorporated into an article and, thus, prevented from release (REACH TGD, Chapter R.5: adaptation of information requirements).

Another area where the notion of very low or negligible exposure has been raised is in the new EU regulation No 1107/2009 concerning the placing of plant protection products on the market that states that “*An active substance (...) shall only be approved if (...) it is not or has not to be classified (...) unless the exposure to humans to the active substance, safener or synergist in a plant protection product, under realistic proposed condition of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1) (b) Regulation No 396/2005*”, i.e. a maximum residue level (MRL) at 0.01 mg/kg food (EU, 2009). No value was provided for non-dietary exposure. However, a recent workshop of the German Institute for Risk Assessment (BfR) recommended that a science-based definition of negligible exposure be defined and suggested to apply a suitable margin of exposure (MoE) or the concept of toxicological threshold of concern (TTC, described further below) (BfR, 2010). Further, the European Crop Protection Agency (ECPA) has proposed a tiered approach to the risk assessment of negligible exposure making use of the TTC concept as a first tier and demonstrating a margin of safety of at least 1000 as a second tier (ECPA, 2010).

The TTC concept has been outlined in several publications (such as Kroes et al, 2004; SCCS, SCHER, SCENIHR, 2012; EFSA, 2012a); a concise overview is to be found in Hennes (2012). A TTC can be applied if human exposure via the skin is expected to be negligible, i.e. as a ‘health-based reference value’ without the need to generate substance-specific data. The TTC approach was primarily developed to derive exposure levels of no concern upon oral exposure. An adaptation for topically applied chemicals has been proposed by Kroes et al (2007), by extrapolating from oral to dermal exposure scenarios. The opinion of SCCS, SCHER, SCENIHR, however, highlights additional work that is needed for route-to-route extrapolation, stating that, “*It is recommended that when applying the TTC approach for cosmetics, an adjusted internal TTC value is defined considering both dermal and oral absorption.*” There are also

developments for specific dermal-TTC values to address sensitisation, although these need still further discussion (Safford, 2008; Safford et al, 2011; Keller et al, 2009).

For effects without threshold (e.g. genotoxic carcinogens), an acceptable risk level, e.g. a derived minimal effect level (DMEL) may be established. The DMEL is described in the REACH TGD, Chapter R.8 as: “...an exposure level that is considered to represent a risk level where the likelihood that effects (cancer) are avoided is appropriately high and of very low concern, acknowledging the fact that for non-threshold carcinogens a dose level without any residual cancer risk cannot be identified.” This is a level whereby the risk of developing cancer could, for example, be expected as low as one in a million.

3.1.5 Generate toxicological data

If there are no available studies and all other non-animal approaches have not been successful in being able to derive a health-based reference value for the substance then it will come to the point where *in vivo* toxicology study data will need to be generated. Unless the only relevant route of exposure is dermal, it is not advocated that a study by the dermal route be the study design of choice for a risk characterisation for dermal exposure due to the technical challenges of repeat-dosing dermally. The orally applied dose route is often more reproducibly absorbed than dermally applied dose. Hence, to reduce data variability, it should be considered whether new toxicology data are to be generated and whether the route of choice for chronic dosing of non-volatile chemical substances would be by oral gavage or dermal application. Such an oral study will yield a NOEL or LOEL for any systemic effects seen and the route used. Corrections can be applied for route to route differences in the next stage of the risk assessment.

The risk characterisation is then largely driven by the extent to which systemic exposure is achieved by the dermal route under the exposure scenario conditions, which in most cases will be significantly lower than the exposure in the study design by the oral route. *In vivo* studies using the dermal route are most appropriate for scenarios with almost exclusively dermal exposure, but repeat-dose dermal studies do not test the intrinsic capacity for a substance to be generically toxic to the body, but already incorporate negligible or reduced systemic delivery in their design. It is possible to use a dermal study to extrapolate to an oral or inhalation scenario provided skin and gut penetration data are available or can be estimated. If skin penetration data are available or can be estimated, it is also feasible to use an oral study to extrapolate to the dermal scenario.

The REACH TGD includes explanations of all the available test methods (*in vitro* and *in vivo*) that can be used to generate appropriate data against each relevant end effect. Some explanations are in the TGD on how repeat-dose toxicity tests should be performed via the dermal route of exposure.

3.1.6 Derive a health-based reference value

There are four routes of deriving a health-based reference value and entering into risk characterisation:

1. A health-based reference value has already been generated and published by a reliable source;
2. toxicology data exists and can be evaluated by a competent toxicologist;
3. exposure-based waiving can be applied and a TTC approach be used;
4. new toxicology data can be generated and evaluated.

Whichever of the above routes has been taken to assess the data relating to human health hazards in a quantitative manner, the end result should lead to a single health-based reference value that uses data for the most sensitive known health effect, using the best toxicological data available to underpin this value. In the risk assessment for systemic effects defined in subsequent sections of this document, the internal dose (in mg/kg/day) derived from a modelled exposure scenario (incorporating a level of skin absorption from an amount of substance applied to a defined area of skin) is to be compared with the health-based reference value, hence for evaluating systemic effects the latter should be derived as a mg/kg/day body burden.

The health-based reference value should incorporate a sufficient level of safety, by the use of appropriate assessment factors e.g. the following equation taken from the REACH TGD illustrates how, in principle, to calculate a dermal DNEL.

$$\text{Route and Endpoint-specific Derived No Effect Level (DNEL)} = \text{NO(A)EL}_{\text{corr}} / (\text{AF}_1 \times \text{AF}_2 \times \dots \times \text{AF}_n)$$

A correction to (in this case) the NO(A)EL (from the pivotal toxicology study) is being made to allow for differences in the route of exposure, and an assessment factor (AF) is being applied to account for uncertainties in the source of the data (REACH TGD Chapter R.8; ECETOC, 2010). The rationale for all correction and assessment factors should be captured. Guidance for the correction factors to apply for route-to-route differences and assessment factors to be applied for different types of uncertainty in the data can be found in the given references.

Care should be taken that considerations for difference in toxicokinetics between routes are only taken into account once in a risk assessment i.e. in the correction of the NO(A)EL or in the modification of the internal dose calculation, but not both.

Dermal DNEL can reflect either the systemic dose causing the effect resulting from the dermal route or the external dose that causes the effect. Both of these can be expressed as mg/kg/day. It is important that this distinction is made otherwise it is possible that an error can occur when

undertaking the risk assessment by applying the same correction factors to in the generation of the DNEL and converting the external exposure to a systemic dose.

Dermal DNEL can also be expressed as mg/cm^2 , but this is more commonly applied to reflect local effects, irritation, corrosively etc.

Dermal DNEL based on extrapolation of oral studies can be considered conservative, particularly for those substances where the dermal penetration is not high as the kinetics of absorption of the substance following dermal dosing will be different to oral administration. For example, a much higher dose is needed to get the same AUC or Cmax following dermal compared to oral. This effect is not considered when simply taking into account difference in per cent absorption between the two routes.

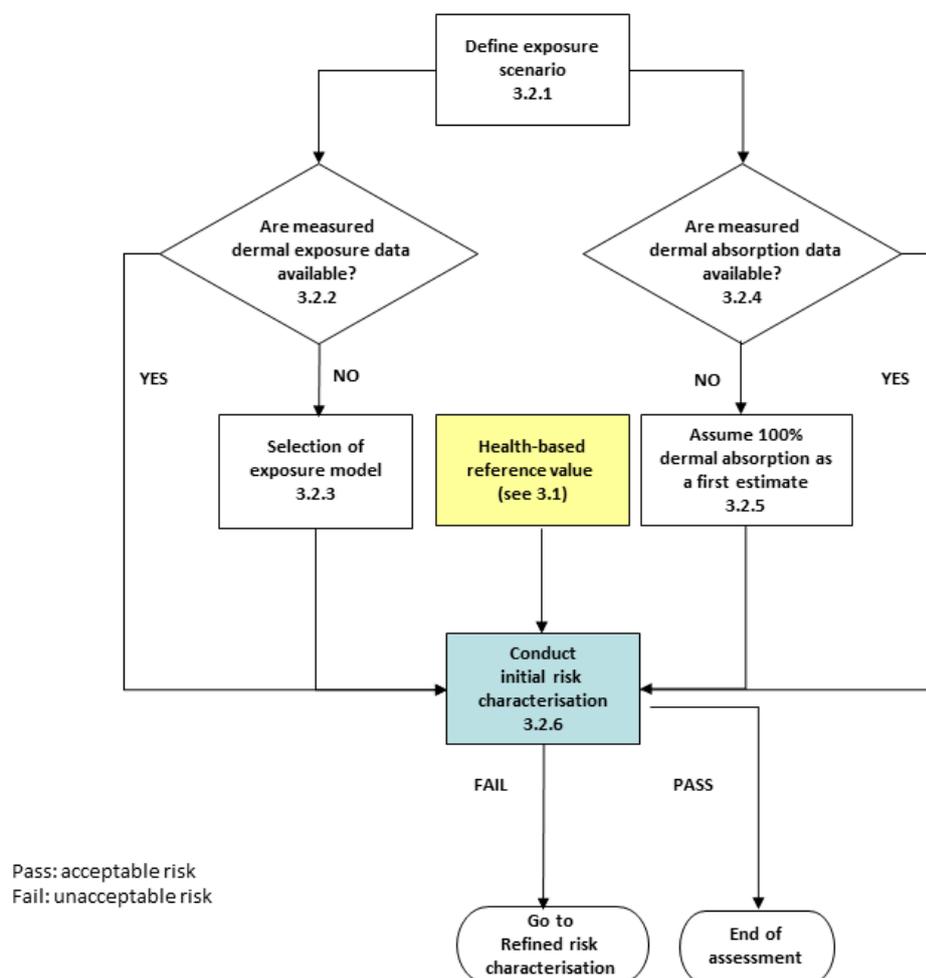
3.2 *Initial risk characterisation*

The objective of decision tree 2 is to determine both the external dermal exposure to a substance and its subsequent absorption through the skin. This allows a health risk characterisation to be performed, provided a health-based reference value as identified by following the process outlined in decision tree 1 (Figure 1) is available.

Risk characterisation is a procedure that allows the comparison of a health-based reference value with the actual systemic dose resulting from an external dermal exposure modified by dermal absorption, i.e. the conversion of an external exposure to an internal dose. The process is outlined in Figure 2.

The availability of data highlighted in Section 3.1 is also relevant to Figure 2. The absence of data specifically in the areas of external dermal exposure can be addressed using exposure models and the options for determining the best estimate of the subsequent dermal absorption. The use of these data in conjunction with a health-based reference value enables a tier 1 risk assessment to be undertaken.

Figure 2: Initial risk characterisation



3.2.1 Define exposure scenario

The importance of understanding the actual exposure scenario for an industrial chemical cannot be understated. This has to be achieved before undertaking any risk characterisation as this allows the accurate identification of any appropriate existing data set for that scenario or the selection of the most relevant exposure model that covers the exposure scenario requiring risk characterisation.

In the EU, specific exposure scenarios have been identified and included in the REACH TGD (part D: exposure scenario building and exposure scenario format).

3.2.2 Are measured dermal exposure data available?

Dermal exposure data can exist as a measurement of the external dose that could reach the skin or alternatively as an internal value obtained through bio-monitoring. The bio-monitoring value would already take dermal absorption into account but would in most instances require additional data to allow interpretation (see section 3.3.8).

External measurements can be obtained by passive dosimetry and/or utilising hand washes. Such data can be obtained on the chemical that requires risk characterisation. But also data obtained from a chemical used in a similar exposure scenario and having similar physicochemical parameters are relevant, as the external dose is considered generic.

Similarity of physicochemical properties has to be argued on a case by case basis. The key parameters are $\log K_{ow}$ and volatility, to a lesser extent, molecular weight and water solubility.

Dermal exposure measurements are usually expressed in amount of material deposited onto the skin (hence units are for instance mg/cm^2 or $\mu\text{mol}/\text{cm}^2$). It is important that the unit chosen can be compared to the unit defined in the hazard assessment of the substance. For specific exposure scenarios, a combination of models may be applied to estimate dermal exposure (Appendix B).

3.2.3 Selection of exposure model

The simplest exposure scenario to begin with is for a single exposure that is not removed. The calculation starts with an estimate of external exposure, i.e. the dose that is deposited onto the skin per incidence of exposure. This is most useful if calculated initially as mg/cm^2 skin exposed. It can be determined by measurement or estimated by modelling. At this point in the calculation, the total surface area exposed (which is scenario-dependent) should also be incorporated, e.g.:

$$\text{External dose (mg/cm}^2\text{)} \times \text{Total exposed surface area (cm}^2\text{)} = \text{Total external exposure dose (mg)}$$

The external exposure dose (mg) can be converted to a systemic dose by using a percentage of the deposited material that could be absorbed across the skin (per exposure over a 24-hour period). Like for the external exposure, this percentage can be either determined by *in vivo* or *in vitro* measurements or estimated by using models.

The selection of an external exposure model is determined by the substance of interest and/or the exposure scenario. For certain classes of substances, specific models have been developed such as the EUROPOEM model for pesticides (Hamey, 2001; Van Hemmen, 2001) and PROMISE for

volatile substances¹. If a model has specifically been validated for a certain substance and/or application, it is in most cases the most appropriate model to apply provided the results are expressed in units that can be used for the risk assessment. For substances and/or exposure scenarios for which specific models are not available, generic models can be used. The EASE model (Estimation and Assessment of Substance Exposure), developed by the UK HSE, has a specific dermal exposure module and is probably the most widely used exposure model as it was recommended in the technical guidance documents for risk assessments under the Existing Substances Regulation (793/93/EC) (EU, 1993). It has also been built into the ECETOC TRA (targeted risk assessment) tool². As a consequence, there is a lot of experience with the model which, however, only predicts the potential exposure rate to hands and forearms (~2000 cm²). The EASE model is known to provide an overestimate of exposure and may be used as a first approximation of dermal exposure (Northage, 2005). If dermal exposure estimated with EASE does not point at a significant risk, the actual risk will indeed be negligible in the vast majority of cases. Other generic models, such as RISKOFDERM and DREAM are semi-quantitative and labour-intensive compared to EASE and less suitable in a first-tier approach (Oppl et al, 2003; Van Wendel De Joode et al, 2003; 2005).

3.2.4 Are measured dermal absorption data available?

Once the initial external dermal exposure has been assessed according to the left-hand side part of Figure 2, the complementary dermal absorption side of the decision tree needs to be explored. Knowledge of the likely degree of absorption of a chemical through the skin is necessary in order to perform a meaningful risk assessment. The ideal situation would be to have dermal absorption data for the chemical concerned. Often, dermal absorption data are generated in rodent studies. It should be borne in mind that rat skin is more permeable than human skin (van Ravenzwaay and Leibold, 2004a, b). If the risk assessment provides an acceptable risk, no further data will be required. Section 3.2.5 indicates the steps to take in the absence of dermal absorption data for the chemical concerned.

3.2.5 Assume 100% dermal absorption as a first estimate

In the absence of dermal absorption data for the chemical of concern a first step would be to perform risk assessment using the worst-case assumption of 100% absorption. This assumption is a REACH requirement but turned out to be an over-estimation in all known cases. If the health-based reference value and/or the dermal exposure estimate result in no systemic exposure of concern, then refinement of a 100% default value in the absence of dermal absorption data is not required.

¹ www.americanchemistry.com/S_ACC/sec_solvents.asp?CID=1529&DID=5769

² www.ecetoc-tra.org

If the assessment indicates a potential health risk using the 100% assumption, there is still a possibility of estimating the degree of dermal absorption based on existing data for a chemical analogue or analogues. The REACH TGD, Chapter R.6, defines a chemical analogue as being a chemical that will possess similar physicochemical, toxicological and/or environmental fate properties to the chemical of interest. Therefore, groups or categories of chemicals can be compiled based upon the hypothesis that the properties of a series of chemicals with common structural features will show predictable trends in their physicochemical properties and/or dermal absorption endpoints. The 'read-across' approach is a method that permits a prediction of a given endpoint for a chemical based upon the information available from another chemical that is judged to be similar such as having common structural subgroups or similar activities. As such the read-across approach can be used to fill data gaps (such as unknown dermal absorption values) in sequences of chemicals that have been grouped together and have displayed a trend for the endpoint of interest. Alternatively, dermal absorption data may be estimated or determined as described in Sections 3.3.4 and 3.3.5/6, respectively.

3.2.6 Conduct initial risk characterisation

The reader is directed to consider as background the general principles of risk characterisation presented in the REACH TGD, part E.

The following are tier 1 tools for estimating dermal exposure (skin loading).

- ECETOC TRA (website: www.ecetoc-tra.org); output for dermal exposure is amount of chemical per cm² of skin.
- COSHH Essentials (Control of Substances Hazardous to Health) (website: <http://www.coshh-essentials.org.uk/>) developed by the Health Safety Directorate, UK.
- RISKOFDERM (website: www.tno.nl; keyword search: 'riskofderm').

For workplace exposures, occupational exposure limits (OEL) may already exist. Under certain circumstances OEL and/or the underlying information used for setting the OEL can be used to estimate a DNEL, or derived no effect level. The DNEL is normally expressed as an external exposure level below which an adverse effect on human health is not expected. For some effects, DNELs by definition cannot be determined, either because the effects are considered non-threshold (e.g. mutagens, genotoxic carcinogens), or typical experimental data on some threshold effects do not normally allow the setting of a DNEL (e.g. sensitisers, corrosives). The reader is further advised to consult the REACH TGD, Chapter R.8: characterisation of dose [concentration]-response for human health for derivation of DNEL.

For most substances, there will only be toxicity data for a single exposure route, and the DNEL values for the other routes, particularly dermal, will have to be generated by means of route-to-

route extrapolation. NOAEL/LOAEL, when based on animal testing, are typically adjusted by assessment factors for interspecies and intra-species variation, differences in exposure (route, duration, frequency), existing dose-response relationships and targeted populations, which provides a ‘margin of exposure’ (MoE) when used to evaluate the dermal exposure route in the workplace.

For human health endpoints, a distinction should be made between effects exerted by a threshold and non-threshold mode of action. For threshold effects, for which a DNEL can be set, the risk characterisation ratio (RCR) is the ratio of the estimated exposure (in this case modelled) and the DNEL; RCR is presented as a new term from REACH. For non-threshold effects (e.g. non-threshold mutagens and non-threshold carcinogens) a no-effect level, and thus a DNEL, cannot be established. However, it may be possible, if data allow, to set a DMEL (derived minimal effect level), a reference risk level considered to be of very low concern. Risk characterisation then entails a comparison between the estimated exposure and the DMEL. In this situation, the principle of RCR may be used by replacing DNEL with DMEL.

When the estimated or modelled (worker) dermal exposure is compared to the health-based reference value (e.g., no-observable-adverse-effect-level (NOAEL), low-observable-adverse-effect-level (LOAEL), ADI, TLV, OEL, DNEL, DMEL), assuming 100% systemic availability of the modelled or estimated dermal dose (Tier 1), the MoE or the RCR can be determined (estimated or modelled [dermal] exposure ÷ DNEL or DMEL). The exposure risk to humans can be considered to be controlled if exposure levels do not exceed the appropriate DNEL or DMEL (RCR <1). However, when RCR >1, the assessment will require refinement.

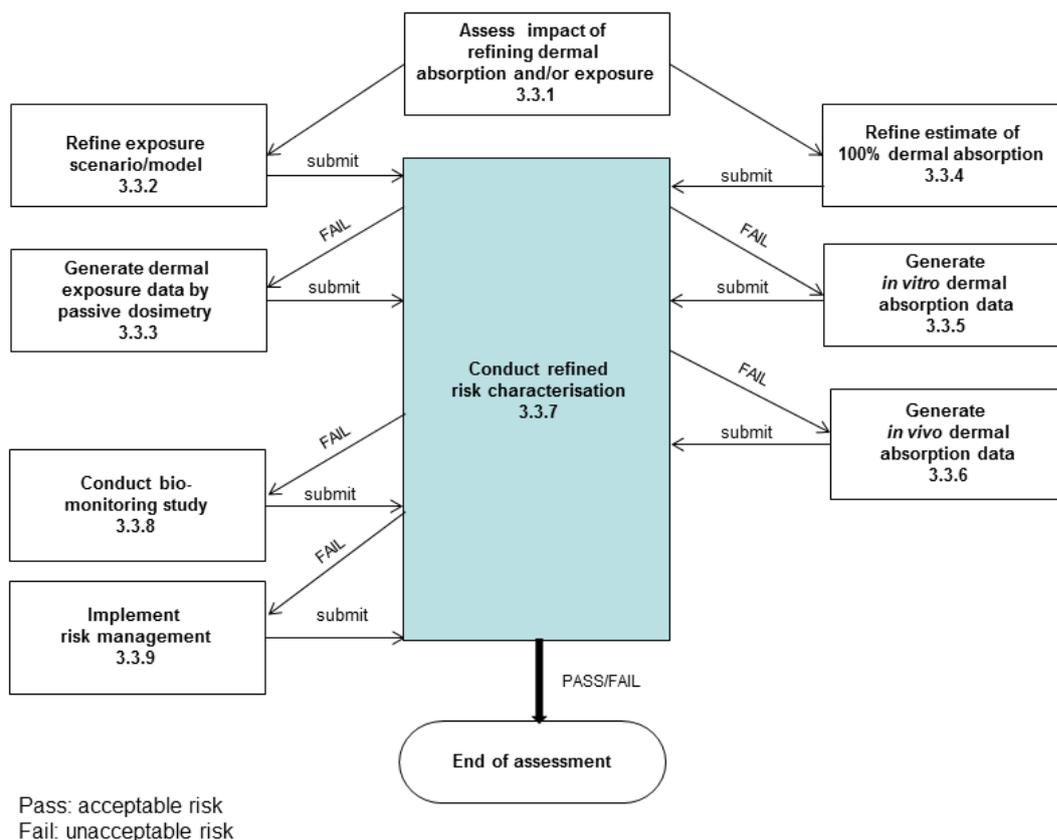
From the REACH TGD, part E: risk characterisation: “When the risk characterisation shows that the risks are not controlled (RCR >1) there are three principal options to iterate the assessment by including more or other information into the assessment: (1) refine the hazard assessment by obtaining more data, which may include the proposal for testing; (2) refine the exposure assessment by ensuring that the exposure estimation is realistic and reflects the use conditions defined in the initial exposure scenario (models or monitoring data can be used to this end); or (3) refine the conditions of manufacture or use (e.g. by introducing more stringent risk management measures (RMM) or changing the operational conditions in the exposure scenario).”

3.3 Refined risk characterisation

Undertaking a refined risk characterisation is not an iterative process but rather a series of solutions that can be implemented based on the likelihood of success. Therefore, Figure 3 is not presented as a decision tree based on a tiered process but rather as a range of options to generate data on either dermal exposure and/or dermal absorption which will lead to a refined assessment

of risk. If these approaches fail, the remaining options are to directly measure the systemic dose resulting from a specific exposure scenario, e.g. bio-monitoring, and/or the implementation of a risk management process; these are also discussed.

Figure 3: Refined risk characterisation



3.3.1 Assess impact of refining dermal absorption and/or exposure

The process is reiterative requiring evaluation of the various options given in Figure 3. Refining the dermal absorption value may indicate that the dermal risk is acceptable or, alternatively, refining the dermal exposure assessment may be undertaken. If both of these approaches individually suggest there is a health risk associated with the dermal exposure, a combination of both approaches may be required.

For example, if a reduction from 100% to 10% in dermal absorption would lead to the conclusion that the exposure does not pose a health risk, it might be considered reasonable to conduct an

in vitro and potentially also an *in vivo* study to assess the actual dermal absorption value. The selection of the appropriate study design is dependent on many factors (ECETOC, 1993a; IPCS, 2006; OECD, 2011). A reduction from 100% to 1% to reach the conclusion that there is no health risk due to dermal exposure might be considered unlikely. However, depending on the physicochemical properties of the substance, this might be possible in some cases.

Refinement of the dermal exposure data could be considered as an alternative approach depending on an assessment of the data behind the model used in Figure 3. Again, the risk characterisation generated via Figure 3 can be used to determine if the generation of dermal exposure data will be sufficient or whether there might be a requirement to use this data in conjunction with dermal absorption data generated with the *in vitro* or *in vivo* study design.

If a review of the impact of generating both dermal exposure and dermal absorption data indicates that there is a possibility that the risk characterisation might fail, then the option exists to generate a direct measurement of systemic exposure utilising bio-monitoring and or implement risk management practices before undertaking the processes outlined in the refined risk characterisation (Figure 3).

3.3.2 Refine exposure scenario / model

Many models are generic and do not reflect the specific scenario in which exposure could occur. Refinement of the exposure assessment can be achieved by ensuring that the exposure estimation is realistic and reflects the use conditions defined in the initial exposure scenario (models). Attention should be paid to the duration of exposure, the amount of a contamination that could occur over the duration of exposure, the distribution of exposure across the worker, and the nature of clothing. Since operators' exposure should be limited by application of engineering controls and personal protective equipment is only the last resort to reduce exposures if engineering controls are not possible or insufficient, models usually do not take the use of personal protective equipment into account. As a consequence, when personal protective equipment is applied in a particular scenario, the dermal exposure as estimated by modelling usually needs to be adapted considering the reduction in exposure by the use of the protective equipment.

3.3.3 Generate dermal exposure data by passive dosimetry

Dermal exposure is in most cases expressed as external exposure, i.e. the net amount of material deposited onto the skin. The most frequently used methods for assessment of dermal deposition include (1) surrogate skin, (2) removal, and (3) visualisation. In addition, one can apply (4) bio-monitoring for dermal exposure assessment, which differs from the other 3 methods in that it

assesses total systemic exposure. Therefore, it is in essence substance-specific but it is not exposure-route specific.

1. Surrogate skin methods deploy methods covering those parts of the skin that are thought most likely exposed, with a layer of material that can absorb the substance under study. After the exposure, the substance is removed from the surrogate skin and analysed. Gloves are most commonly used in this approach. If normally no gloves are used, gloves from a material that is suitable to absorb the substance under study can be used. If normally protective gloves are used, special (thin) gloves, usually cotton gloves, are worn underneath the normally used gloves. If the exposure is not limited to the hands and underarms, swabs or rinses from clothes may be used to assess external exposure or a number of patches, which are placed on representative parts of the skin.
2. Removal of the substance deposited onto the skin for quantification is usually performed by simply washing the skin with water containing detergent or a suitable solvent solution, collection of the washings and subsequent quantitative assessment of the substance in the washings. A potential limitation to this approach sometimes raised, is the potential underestimation of the chemical already absorbed into the skin. This concern can be addressed by providing data that illustrates the washing efficiency of the process. One approach is to utilise the data generated in an *in vitro* dermal absorption study, where the material washed off the skin at the end of a typical work day (6 to 8 hours) is determined, a correction factor can then be applied, if required.
An alternative option, which allows determination of the amount of material absorbed in the *stratum corneum* of the skin, is tape stripping. In this method, the *stratum corneum* is removed using adhesive tape which is stuck unto the skin and subsequently removed for determination of the substance under study in the adhesive layer. This may be repeated several times on the same spot of the skin which allows obtaining some additional insight in the distribution of the substance in the *stratum corneum* and the extent of dermal absorption and penetration.
3. In a limited number of cases, dermal deposition of a substance can be determined by visualisation. This method is applicable to substances that can be easily visualised through their specific physical characteristic of the substance under study (e.g. fluorescence) and to specific, usually occupational, situations (e.g. spraying of pesticides) where a specific visual marker can be added to the substance under study.
4. Bio-monitoring is dealt with separately in section 3.3.8 (see below); it has several other advantages and practical implications.

3.3.4 Refine estimate of 100% dermal absorption

Refinement of the default 100% value can be attempted using three approaches. The success of the approach chosen will depend on the availability of existing data.

a) Physicochemical parameters

Guidance on the use of two physicochemical parameters (MW and $\log P_{ow}$) is presented in an EU guidance document on dermal absorption (EC, 2004). As a first step, it is possible to assume 10% dermal absorption, if the molecular weight is > 500 and $\log P_{ow} < -1$ or > 4 . This excludes a significant number of chemicals and makes no allowance for the physical state of the chemical, solid or liquid, neat or in solution etc. A more recent guidance document was issued by the European Food Safety Authority that indicated that the default value of 25% may be used for plant protection products containing $> 5\%$ active substance and whilst a value of 75% can be used for products or in-use dilutions containing $\leq 5\%$ active substance (EFSA, 2012b).

Guidance on further refinement for the acquisition of this type of data is given in Appendix C.

b) Read-across

This approach is potentially of high utility as it can be applied to homologous series or classes of chemistry. At the simplest level it can be applied to chemical with similar physicochemical properties and functional groups, but it should only be attempted when data have been collected for all chemicals using the same vehicle.

c) QSARs

It is possible to use the saturated water concentration of the molecule and the K_p (which requires an estimate of the K_p value at this stage) to calculate an estimate of maximum flux, allowing for any effects from the vehicle. The next step would be the use of a more complete mathematical model with diffusion coefficients and partition coefficients to obtain a best estimate of the flux and dose for the likely occupational exposure concentration (i.e. finite dose).

The Skinperm model (V 9.0 was released in 2010) has been well validated and has proven to be very useful in predicting the penetration and permeation of substances (Wilschut et al, 1995; ten Berge, 2009). The model is based on two QSARs, one describing the human aqueous permeation coefficient of the *stratum corneum*, primarily based on the octanol/water partition coefficient ($\log K_{ow}$) and the molecular weight, and the other describing the *stratum corneum*/water partition coefficient (ten Berge, 2009; Vecchia and Bunge, 2002a,b). The model requires basic input (molecular weight, octanol/water partition coefficient and water solubility) and is an open source programme running under MS Excel. It is easy to operate and allows simulation of several practices and conditions (e.g. time of exposure, evaporation from the skin of volatile substances, loss due to sloughing of the *stratum corneum* etc.).

Further information on QSARs for skin permeability is given in Appendix D.

3.3.5/3.3.6 Generate *in vitro* / *in vivo* dermal absorption data

In the event that the refinement of the default dermal absorption value(s) described in the section above still indicates an unacceptable risk, the generation of appropriate dermal absorption data should be considered. The first step regarding the use of *in vitro/in vivo* dermal absorption studies should be to try and determine whether the expected refinement is likely to provide dermal absorption values that will improve the risk assessment. The decision as to which studies to perform in order to measure the dermal absorption of a chemical will also depend, to some extent, upon where the chemical is going to be registered and under what legislation. In principle, it is relatively easy to determine the probability of obtaining a useful answer by running the risk assessment using the chosen exposure model and observing the effect of changing the dermal absorption value. Once the level of dermal absorption required to pass the risk assessment has been determined, the next step would be to decide whether such a level is likely to be provided by a study. The use of chemical analogue groups and a read-across approach may help in this decision making. There may also be the possibility of using an existing QSAR model in order to predict the likely dermal absorption of the chemical based upon its physicochemical properties. In the EU, the first study that is likely to be considered would be the *in vitro* study using human skin (generally following the OECD 428 test guideline and the OECD guidance document; OECD, 2004c, 2011).

If the dermal absorption value obtained from the *in vitro* human study is not sufficiently low to allow the risk assessment to pass, there is the possibility of performing an *in vitro/ in vivo* rat study. The value of performing an *in vivo* study is the potential that it will be possible to demonstrate the fate of the material in the *stratum corneum*. In some cases it is possible to demonstrate that the dermal exposure does not lead to the absorption of the material found in the *stratum corneum* because it either does not penetrate deep enough into the *stratum corneum* and is lost through sloughing of the dead skin cells or that the material is incapable of penetrating the more hydrophilic viable epidermis and is 'trapped' in the *stratum corneum* until lost as the *corneocytes* are lost. The inclusion of in an *ex vivo / in vitro* rat skin study facilitates the use of the so called 'triple pack' or 'parallelogram' approach the estimated *in vivo* human absorption value can be calculated using the following formula:

$$\text{Human}_{(\text{in vivo est})} = \text{Rat}_{(\text{in vivo})} \times \text{Human}_{(\text{in vitro})} / \text{Rat}_{(\text{in vitro})}$$

A selection of guidelines on how to conduct such studies is given in Appendix E.

3.3.7 Conduct refined risk characterisation

If an initial risk characterisation results in a risk characterisation ratio (RCR) > 1 (Figure 2), refinement of either the dermal exposure (amount of chemical deposited per area of skin), and/or the assumption of 100% dermal absorption can be modified by collection of actual data. Collection of actual data assumes that operation control and/or risk reduction measures were also unsuccessful at deriving an acceptable evaluation (RCR < 1). Based on these refinements, if the dermal exposure levels are lower than the original estimates, assumption of 100% absorption of this new skin exposure value could result in an acceptable MoE or RCR. Additionally, replacement of the assumption of 100% dermal absorption with actual measured data from either finite dose or infinite (permeability coefficient, Kp) *in vitro* dermal evaluations, or workplace bio-monitoring studies, could also yield systemic levels giving an acceptable MoE or RCR.

3.3.8 Conduct bio-monitoring study

Bio-monitoring, which denotes in this context the assessment of internal dose or body burden, assesses the total absorbed amount of the substance under study regardless the route of exposure. Hence bio-monitoring reflects all routes of exposure (including dermal, inhalation and oral exposures). For substances with negligible vapour pressure and which are not dusty or aerosolised, bio-monitoring is a highly suitable metric for dermal exposure. In addition, in occupational situations where respiratory protection is effectively applied bio-monitoring can be used to assess dermal exposure. In other situations, where other routes of exposure than dermal cannot be excluded, bio-monitoring can still be useful to estimate the contribution of the dermal component in the total exposure if an estimate of other exposure routes is available or as an absolutely worst-case estimate. When the relative contribution of various exposure routes is unclear, bio-monitoring is highly useful as a first tier exposure assessment in an occupational setting. If the bio-monitoring results indicate that total exposure is below the health-based reference value (e.g. a BEI or a BAT value), there is no health risk and therefore no immediate need to further investigate the various sources of exposure (Boogaard, 2008; Boogaard et al, 2011). At present, validated bio-monitoring methodologies are available for a limited number of substances only.

3.3.9 Implement risk management

In the context of this guidance, risk management is defined as measures that control the emissions of a substance and/or exposure to it, thereby controlling the risks to human health. In terms of dermal exposure this refers to separating the potential dermal exposure from the skin either by engineering controls, potentially redesigning the process which results in elimination or reduction of potential dermal exposure, or by personal protective equipment.

If the revised input data to the risk assessment, using all available information (modelled or measured) leads to an unfavourable risk assessment, there may be value in generating new data relevant to the specific risk assessment scenario, to gain more accurate knowledge and hence refine the risk assessment.

One of the tools uses ‘skin notation’. Basically, a skin notation indicates that a chemical has the potential to add to the systemic burden upon skin contact (ECETOC, 1993a). Hence, a skin notation is a strong indication that dermal exposure may lead to health risks. Skin notations are reserved for chemicals shown to elicit toxicity following dermal exposure and when it is felt that adherence to OELs is insufficient to protect the safety and health of workers. According to ECETOC (1993a), skin notations should be assigned when the amount of chemical absorbed by both hands and lower arms (approximately 2000 cm²) for one hour is expected to contribute >10% to the systemic dose, compared to the amount absorbed via inhalation exposure at the OEL during a full work day (assuming that 10 m³ air is inhaled during an 8-hour workday and that 50% absorption of the inhaled air). Below is an example of the skin absorption time to the OEL (TLV) for acetonitrile (ACN) using measured dermal penetration data.

Acetonitrile, which has an ACGIH-TLV of 20 ppm (33.6 mg/m³; skin notation) as an average over 8-hour workday (TWA), and assuming a worker breathes in 10 m³ of air in 8 hours (336 mg ACN inhaled), with 50% retention of the inhaled ACN, the total (calculated) systemic dose of ACN would be estimated at 168 mg. Steady-state uptake of neat ACN applied to human skin *in vitro* has been reported to be 143 µg/cm²/hour (Fasano and McDougal, 2008). Given the steady-state uptake and an exposure area of 2000 cm² (hands and arms) it would take about 35 minutes to achieve an equivalent internal dose at the TLV via the dermal route [(143 µg/cm²/hour × 2000 cm²) × 0.587 hours = 168 mg ACN]. Although this example assumes continuous exposure of the hands and arms to the neat material, which may or may not be a practical workplace scenario, it nonetheless illustrates the potential contribution of the dermal exposure route to the systemic burden when unprotected skin comes into contact with acetonitrile.

4. CASE STUDIES

4.1 Colourant

This example looks at a semi-permanent water soluble ($\log P_{ow} -1.5$) non-oxidative hair colouring substance imported into the EU at 2.5 t/y. For the purposes of REACH, a search for existing data has revealed acute toxicity data indicating an LD_{50} of 2000 mg/kg; LLNA data indicating the substance is non-sensitising and *in vitro* mutagenicity data (Ames and *in vitro* chromosome aberration assay) indicating it is not mutagenic. There is a 28-day study on the substance and there are also 28-day repeat-dose studies on three ‘similar’ chemicals in a chemical category.

Derivation of a health-based reference value (according to 3.1 / Figure 1)

Has a health based reference value previously been derived (3.1.1)? NO

Are toxicological data available (3.1.2)? YES

Acute toxicity data $LD_{50} = 2000 \text{ mg/kg}$
[Klimisch score 2 - Reliable but with restrictions, study performed in 1975; pre-GLP]

Sensitisation (Local Lymph Node Assay) = Negative
[Klimisch score 1 – Reliable – study performed in 1997; OECD guideline 429]

Mutagenicity (I Ames and *in vitro* IVC) = Negative
[Klimisch score 1 – Reliable – study performed in 1990; OECD guideline 471]

28-day sub-chronic repeat-dose oral study in rat Oral NOEL = 500 mg/kg/day
[Klimisch score 1 – Reliable. OECD guideline 412. Doses tested 0, 250, 500, 1000 mg/kg/day. Effects on the liver and changes in clinical chemistry seen at the top dose only.]

Is read-across or QSAR possible using data on similar substances (3.1.3)? YES

Three chemically ‘similar’ substances have reliable 28-day systemic toxicology data indicating oral NOEL doses ranging between 500-750 mg/kg/day. These studies provide additional weight of evidence to corroborate the NOEL for the-28 day study on the substance of interest but are not required for deriving a health-based reference value. The best systemic toxicity study to use is the 28-day oral repeat-dose study for the substance itself, with a NOEL of 500 mg/kg/day.

Derive a health-based reference value (3.1.6):

The oral NOEL is 500 mg/kg/day. This is the amount of substance administered into the gut as an external dose in animals that shows no effects. Accompanying ADME data for this substance showed that 50% of the oral dose was absorbed through the gut and into systemic circulation. The systemic NOEL, in terms of a circulating daily body burden, is therefore 50% of 500 mg/kg/day = 250 mg/kg/day.

In relation to human risk assessment, there may be some uncertainty about this NOEL from a rat study being relevant for man; the rat may have significant toxicodynamic and toxicokinetic differences that lead to interspecies differences in toxicity. So the NOEL is divided by 10 to exercise some degree of precaution about this uncertainty and lack of knowledge. Similarly, inter-individual differences may occur in the toxicokinetics and toxicodynamics between human individuals, so the NOEL is divided by a second factor of 10.

The most appropriate 'health-based reference value' for input into a dermal risk assessment is therefore a NOEL of 250 mg/kg/day divided by an uncertainty factor of 100 = 2.5 mg/kg/day.

Initial risk characterisation (according to 3.2 / Figure 2)

The most common occupational dermal exposure scenario for this hair colouring substance is for hairdressers. Exposure of hairdresser's hands to hair dyes is not always effectively controlled, but is often to low levels only as formulated in the final product. Even though gloves are recommended, they are not always used and exposure to the substance could be many times in a working day. The hair dye is neither a sensitiser nor a mutagen.

A dermal risk assessment scenario for the hairdresser is as follows:

Define exposure scenario (3.2.1):

A hairdresser uses 50 g product containing 1% hair dye in a hair product formulation (worst case - squeezed directly into the hands without the use of gloves per treatment session).

Are measured dermal exposure data available (3.2.2)?

There are no actual measured data on exposure in this context, but estimations can be made as follows.

Selection of exposure model (3.2.3):

A simple exposure calculation can be done in the first instance. The hair dye is used at 1% in the formulation. The hairdresser uses approximately 50 g of product per application neat as massaged into the customer's hair. So, per application, 0.5 g of dye is exposed to both hands (for a 5-10 minute period of time).

Are measured dermal absorption data available (3.2.4)?

There are no actual absorption data on the substance.

Assume 100% dermal absorption as a first estimate (3.2.5):

In the first instance, assuming 100% of the external exposure dose could be absorbed and not considering any skin absorption kinetics for the impact of short duration frequency, this would mean that 0.5 g has the potential to be systemically absorbed.

Conduct initial risk characterisation (3.2.6):

If the hairdresser weighs (on average) 60 kg, this would lead to a body burden per application of $0.5 \text{ g} / 60 \text{ kg} = 8.3 \text{ mg/kg}$ per application. Multiplied by the number of times a hairdresser may dye a customer's hair in a day to calculate a daily body burden, and assuming the hairdresser uses the product on average 5 times a day, this would lead to a body burden of 42 mg/kg/day.

Dividing the initial exposure estimate (42 mg/kg/day) by the health-based reference value of 2.5 mg/kg/day yields a concentration ratio of 16.8. This is significantly greater than 1 and hence the risk assessment requires refining to reflect a more realistic skin absorption parameter.

Refined risk characterisation (according to 3.3 and Figure 3)

It would be beneficial to refine the skin absorption value from 100% to a lower value as it is likely that in reality skin absorption is much lower from this exposure scenario.

Refine estimate of 100% dermal absorption (3.3.4):

It is highly unrealistic to assume 100% skin absorption for the hairdresser in this case, and in the first instance, this is the simplest parameter to refine without experimental data. The molecular weight of the dye is $> 500 \text{ g/mol}$ and it is water soluble ($\log P_{ow} -1.5$). This is not optimal for skin penetration; thus it is unlikely to significantly penetrate the skin. Hence, as per guidance in reference EC (2004), 10% skin absorption could be considered a precautionary estimate.

Conduct refined risk characterisation (3.3.7):

Assuming 10% absorption, the body burden via skin would be 4.2 mg/kg/day. Dividing the revised exposure estimate (4.2 mg/kg/day) by the health guidance value of 2.5 mg/kg/day yields a concentration ratio of 1.68. As this is still above the target ratio of 1, further refinement is needed.

Refine exposure scenario/model (3.3.2):

The majority of the hair dye will be distributed over the customer's hair and will be absorbed by the hair rather than through the hairdresser's hands. Also, the contact time of the hairdresser's hands with the hair colouring will be of the order of 10-15 minutes maximum per application and will be diluted with water when washing off. An in-use study was performed which estimated that the amount of product remaining in contact with the hands during a 10-15 minute application was at worst 5 g (compared to the 50 g used per application). Hence, the exposure estimate can be divided by 10 to account for this.

Conduct refined risk characterisation (3.3.7):

The hands are only exposed to a worst-case estimate of 5 g of product containing 1% hair dye = 50 mg per application, divided by an average body weight of 60 kg = 0.83 mg/kg/application. Assuming five applications per day = 4.2 mg/kg/day and that 10% absorbs through the skin, the body burden via skin is 0.42 mg/kg/day.

Dividing the revised exposure estimate (0.42 mg/kg/day) by the health-based reference value of 2.5 mg/kg/day yields a concentration ratio of 0.168, which is an acceptable risk characterisation ratio.

4.2 Boric acid for spa use

This case study provides a risk characterisation for dermal exposure to a spa care product that contains boric acid (H_3BO_3 ; CAS no. 10043-35-3). The toxicity associated with boric acid (and boron containing chemicals) is expected to be similar based on boron equivalents (US EPA, 2004a); boron represents 17.84% of boric acid by weight. Per the current harmonised EU classification (Annex VI Table 3.1) boron has been assigned reproductive hazard category 1B (H360 may damage fertility or the unborn child).

Derivation of a health-based reference value (according to 3.1 / Figure 1)

Has a health-based reference value previously been derived (3.1.1)?

Yes. The most conservative health-based reference value for boric acid (0.096 mg/kg/day) is presented in ECHA's Committee for Risk Assessment document (ECHA, 2010) entitled 'Opinion on new scientific evidence on the use of boric acid and borates in photographic applications by consumers'. The health-based reference value was derived by first selecting the lowest NOAEL available – in this case, an oral developmental study with a NOAEL of 9.6 mg/kg/day was selected as the point of departure. The NOAEL was adjusted by applying an assessment factor of 10 for interspecies and another factor of 10 for intra-species sensitivity yielding a health-based reference value of 0.096 mg/kg/day. By contrast, the current dermal DNEL in the boric acid dossier at the ECHA website for general population, long-term exposure, systemic effects, is 196 mg/kg/day, which is likely an external dose based on the systemic oral DNEL of 0.98 mg/kg and an assumption of 0.5% dermal bioavailability ($0.98 \div 0.005 = 196$). However, this derivation cannot be confirmed. Therefore, the health-based reference value of 0.096 mg/kg/day will be used for this exercise.

Initial risk characterisation (according to 3.2 / Figure 2)

Define exposure scenario (3.2.1):

Boric acid is applied as a pH buffer and as a biocide agent. In this example, a typical consumer scenario requires adding two pounds of product (908 g) to 300 gallons of spa water (1140 l), which yields a boric acid concentration of 0.781 g/l (approximately 0.8 mg boric acid/ml; 0.14 mg boron/ml). Whole body immersion in the spa for approximately one hour will be evaluated.

The following is guidance from US EPA, Dermal Exposure Assessment, Principles and Applications (1992), page 5-6: "*Skin permeability varies with location on the body due to differences in quality and thickness of the stratum corneum. Lipid content, structure, and other factors may also account for differences. Most parts of the body (abdomen, forearm, forehead, back) have a stratum corneum thickness of 10-16 μ m. Human skin from these areas, which makes up a majority of the surface area, would be more representative of whole body exposure during bathing or swimming than areas with much thicker skin (i.e. palm, sole) or thinner skin (i.e. scrotum)*". Further, total adult (whole) body surface areas can vary from about 17,000 cm² to 23,000 cm². The mean whole body surface area is reported by EPA to be approximately 20,000 cm² and this value will be used in this example.

Permeability is inversely proportional to the thickness of the *stratum corneum*. However, thickness of the *stratum corneum in vivo* and *in vitro* is positively correlated with the relative environmental humidity and degree of hydration of this layer. Therefore, one would expect well-

hydrated skin to be less permeable than relatively dry skin as a result of its increased thickness. This, however, is not generally the case. As a rule, hydration increases the permeability of skin for most compounds. Therefore, there is an increased potential for percutaneous absorption for scenarios such as bathing, swimming, or showering where the skin is well hydrated.

Are measured dermal absorption data available (3.2.4)?

Yes. Dermal absorption from liquids during continuous exposure scenarios is best described by the use of a concentration-normalised (calculated) permeability coefficient (Kp) rather than by using per cent or fractional absorption based on finite dose experiments. Previous *in vitro* dermal testing with human skin mounted in a static diffusion cell model, using a 0.05% aqueous dilution of boric acid, an infinite dose, and a test-model temperature of 32°C (i.e. skin surface temperature), yielded a steady-state penetration rate (as boron) of 0.25 µg boron/cm²/hour with a calculated Kp of 5×10^{-4} cm/hour (Wester et al, 1998). Further, in a human study, volunteers exposed dermally to a 5% boric acid solution for 24 hours resulted in a dermal flux of 0.009 µg boron/cm²/hour (based on urinary excretion rate of boron) with a calculated Kp of 1.9×10^{-7} cm/hour. In these examples, the flux of boron was noted to >1000-fold faster *in vitro* (5×10^{-4} cm/hour) than *in vivo* (1.9×10^{-7} cm/hour). Therefore, use of the *in vitro*-derived Kp (5×10^{-4} cm/hour) would be a conservative approach to evaluating the risk to humans from dermal exposures to aqueous solutions of boric acid.

Conduct initial risk characterisation (3.2.6)

The human *in vivo* dermal (absorbed) dose of boric acid (as boron) resulting from whole-body exposure for this spa scenario can be estimated using the following equation (from Roberts and Walters, 1998).

$$Q = Kp \times A \times C \times (T-t)$$

Where Q is the amount of chemical absorbed (mg), Kp is the permeability coefficient (cm/hour), A is the skin exposure area (cm²), C is the concentration of the chemical in the aqueous dilution (mg boron/cm³), T is the exposure time (hours), and t is the lag time, or time required for the boric acid (as boron) to cross the skin barrier. In general, if the lag time is greater than the exposure interval, the systemic availability of the chemical is likely to be limited. The lag time will be considered 'zero hours' for these evaluations.

Durrheim et al (1980) have shown that dermal absorption rates can increase with an increase in temperature, and Liron and Cohen (1984) have reported as much as a 2-fold increase in Kp *in vitro* with a temperature increase from 37°C to 50°C. In general, a 10°C increase in temperature can result in a doubling of skin permeability (US EPA, 1992). Therefore, in situations where the temperature of skin is elevated to approximately 40°C, as would be

encountered under typical spa use conditions, this evaluation will include penetration of boron from spa water at an elevated temperature.

The values used and results for the initial risk characterisation are presented in Table 1 for two scenarios:

Scenario A – normal skin surface temperature.

Scenario B – elevated skin temperature, typical of spa use conditions.

Table 1: Risk characterisations following whole-body exposure to boric acid under typical spa use conditions

Scenario	Kp (cm/hour)	Skin area ^a (cm ²)	Exposure time (hour)	Boric acid concentration ^b (mg boron/ml)	Total absorbed (Q) (mg boron)	Body weight (kg)	Dermal dose (mg/kg)	HBV ^c (mg boron/kg)	RCR ^d
A	5×10^{-4}	20,000	1.0	0.14	1.40	60	0.02	0.096	0.2
B ^e	1×10^{-3}	20,000	1.0	0.14	2.80	60	0.05	0.096	0.5

^a US EPA (1992), mean whole-body exposure area for an adult

^b $0.8 \text{ mg boric acid/ml} \times 0.1748 = 0.14 \text{ mg boron/ml}$

^c Health-based reference value, 0.096 mg/kg/day (NOAEL \div AF of 100)

^d Dermal dose \div Health-based reference value

^e For scenario B, Kp is 2-fold scenario A to account for an increase in temperature

Under typical spa use conditions (scenario B – elevated temperature) with a total immersion time of 1 hour, whole body exposure to a 0.8 mg/ml solution of boric acid (0.14 mg boron/ml) is estimated to result in a RCR of less than 1. Although the RCR values for both scenarios are only slightly less than ‘1’, the *in vitro*-derived Kp value and further adjustment to account for the increase in temperature makes this assessment conservative.

4.3 Occupational handling of 4,4'-methylenedianiline

Derivation of a health-based reference value (according to 3.1 / Figure 1):

4,4'-Methylenedianiline (MDA) was evaluated under the Existing Substances Regulation, reported under an EU risk assessment report (EC, 2001). It is classified as: Carc. 1B; H350, Muta. 2; H341, STOT SE 1; H370**, STOT RE 2*; H373**, Skin Sens. 1; H317, Aquatic Chronic 2; H411 under CLP.

This risk assessment is based on the health effects observed following oral exposures in rodents. However, similar effects (Epping jaundice) to those observed in animals were also observed in humans following accidental exposure to MDA. Based on the results from a two-year chronic repeat-dose toxicity rat study, an oral LOAEL of 9 mg/kg/day was selected for quantitative risk assessment. Applying an assessment factor of 3 to derive a NOAEL and an assessment factor of 10 for species differences, a reference value of greater than 40 mg/day for chronic systemic, non-neoplastic effects in humans following dermal exposure was derived (EC, 2001). This value is considerably lower than the dermal value calculated for neoplastic effects (> 250 mg/day).

A dermal NOAEL was not reported in the EU risk assessment report (EC, 2001). However, similar effects were found following dermal exposure, which indicates that dermal exposure plays an important role in systemic toxicity. The available data from an acute dermal toxicity study in mice demonstrate a NOEL for the most sensitive toxicological endpoint (increased in liver weight) in the mouse of 84 mg/kg/day. The available data from an acute dermal toxicity study in rabbits demonstrate a LOAEL for hepatic bile duct hyperplasia and hepatic necrosis and cirrhosis of 700 mg/kg/day (Rosemond and Lladós, 1998). The EU risk assessment report suggests a reference value of greater than 140 mg for acute systemic effects in humans following dermal exposure (EC, 2001).

Bos et al (1998) derived a dermal OEL based on the potential neoplastic effects of MDA. The daily internal MDA dose associated with the reference values of 4:1,000 (representing an annual risk of an additional cancer case of 1 in 10,000 assuming a working life of 40 years) is 16 mg.

Initial risk characterisation (according to 3.2 / Figure 2):

Define exposure scenario (3.2.1):

The study by Brouwer et al (1998) provides an initial risk characterisation following dermal exposure during occupational handling of an epoxy resin hardener used in a fibreglass process. The resin hardener contains 12% MDA by weight (120 g/kg). The following 'worst-case' scenario applies to the occupational handling of the epoxy resin hardener under these circumstances:

1. Workers are acutely exposed to the epoxy hardener during specific phases of a construction process. Workers are not repeatedly or chronically exposed.
2. Workers wear gloves during the handling of the resin hardener or during the mixing and handling of the resin.
3. Accidental spillage of the resin hardener onto the hands and forearms is common during the measuring, mixing and clean-up processes. Workers often rub or wipe areas of the skin that

have come into contact with the hardener in an attempt to remove it. This process tends to spread the hardener over a larger area of skin.

4. Assuming an extreme worst-case scenario, both forearms and hands would potentially be covered with the resin hardener. This represents approximately 2000 cm².

Are measured dermal exposure data available (3.2.2)?

1. Dermal exposure was assessed in this study by (1) hand washing and (2) determining the amount of MDA collected on cotton gloves that were worn underneath the normally used gloves. The workers were divided in two groups: during one week the first group applied hand washing and the second group cotton gloves; during the subsequent week the first group applied cotton gloves and the second group hand washing.
2. Concomitantly, bio-monitoring (24-hour urine collection) was applied in both groups during both weeks.
3. Average daily hand wash and glove monitoring results ranged from 81 to 1762 µg MDA and from 84 to 1783 µg MDA, respectively. The amount was related to the job title.
4. Urinary MDA levels ranged from 2 to 99 µg/g creatinine. Again, the amount was related to job title.
5. Bio-monitoring and hand wash data showed a very strong correlation ($R^2 = 0.94$).
6. The data obtained with the cotton gloves indicate an over-estimation of actual dermal exposure.

Conduct initial risk characterisation (3.2.6):

In the study by Brouwer et al (1998), the highest daily dermal exposure value measured was approximately 4 mg, i.e. about 25% of the external dermal occupational exposure limit as suggested by Bos et al (1998). This value is lower than the reference values suggested by the EU.

Based on the dermal OEL of 16 mg and assuming that 8% of the absorbed MDA is excreted in the urine (Brouwer et al, 1998), a biological limit value for 24-hour urine samples of 1.28 mg MDA can be derived. The highest concentration of MDA found in the workers was 0.25 mg, approximately 20% of the dermal OEL.

The values of the bio-monitoring and the hand washing correlate well and suggest that dermal exposure is the major (if not the only) route of exposure. The results also indicate that the occupational exposures to MDA in this glass fibre process are sufficiently controlled.

4.4 Glycol ethers – 2-butoxyethanol

Glycol ethers form a group of highly versatile solvents with very specific properties that vary with the glycol used (ethylene glycol or propylene glycol) and the linked alcohols. Most glycol ethers can penetrate the skin fairly well. Since this has been recognised, biological monitoring methodology has been developed for several glycol ethers.

Derivation of a health-based reference value (according to 3.1 / Figure 1)

2-Butoxyethanol (2-BE) was evaluated under the Existing Substances Regulation and reported under an EU risk assessment report (EC, 2008). It is classified as: Xn; R 20/21/22, Xi; R 36/38 under the DSD, which translates to [Acute Tox. 4*; H332], [Acute Tox. 4*; H312], [Acute Tox. 4*; H302], [Eye Irrit. 2; H319], [Skin Irrit. 2; H315] under CLP. In Commission Directive 2000/39/EC (EC, 2000), the occupational exposure limit for 2-BE is 98 mg/m³ (20 ppm) as 8-h time-weighted average, with a short-term exposure limit (15 min) of 246 mg/m³ (50 ppm); in addition, 2-BE has a skin notation.

The risk assessment is based on haematological effects which were seen primarily in rats following oral or inhalation exposure, but also in mice and rabbits albeit at a much lower severity. Humans appear to be less sensitive than rodent species for the haematological effects. In dermal application studies in rabbits, the animals were dermally exposed to 0, 45, 225, 450 or 900 mg/kg of 2-BE (with occlusion) for 6 hours/day, for five consecutive days, followed by two days of no exposure and another four consecutive days of dermal administration (EC, 2008). The NOAEL was 450 mg/kg. In a GLP repeat-dose toxicity study in rabbits, the animals were dermally exposed for 6 hours/day, 5 days/week for 13 weeks, to 0, 10, 50 or 150 mg/kg (with occlusion). The NOAEL was 150 mg/kg (the highest dose tested). In a limited study in mice, the animals were dermally exposed to 0, 100, 500, 1000 or 1500 mg/kg for four consecutive days. The NOAEL was reported as 1000 mg/kg (EC, 2008). Following oral exposure in rats NOAELs of 38 and 69 mg/kg were reported for 90-day feeding studies and drinking water studies, respectively. In mice a NOAEL of 223 mg/kg was found in a 14-week drinking water study (EC, 2008), suggesting that dermal absorption rates are much lower than oral absorption rates. It was estimated that the dermal absorption rate of 2-BE was 30% for neat liquid and 39% for vapour, and the NOAEL in rabbits of > 150 mg/kg was taken forward for the risk assessment (EC, 2008).

The value of 150 mg/kg can be taken as a conservative starting point to derive a health-based reference value. Considering that humans are less sensitive to the toxicological endpoint (haematotoxicity) an interspecies assessment factor is not necessary. Application of an

assessment factor of 5 to account for intraspecies differences yields a health-based dermal reference value of 30 mg/kg.

Initial risk characterisation (according to 3.2 / Figure 2)

Define exposure scenario / Are measured dermal exposure data available? (3.2.1 / 3.2.2):

2-BE is used to formulate paints for various coating and varnishing applications. Dermal exposure studies, however, are not available.

There are several general methodologies and models available for the estimation of dermal exposure. In the EU health risk assessment of 2-BE, EASE and RISKOFDERM were applied for the estimation of dermal exposure during the formulation of products with 2-BE (EC, 2008). EASE estimates the external exposure to range from 42 to 420 mg/day for neat 2-BE, which was considered a worst-case approach. RISKOFDERM, however, estimates much higher values (3300 and 11000 mg/day for neat and diluted products, respectively). This is probably due to the fact that RISKOFDERM was validated using 2-(2-butoxyethoxy)ethanol assuming that all glycol ethers have similar properties (Van Hemmen et al, 2003).

Gijsbers and co-workers estimated potential hand exposure to an analogous but less volatile glycol ether 2-(2-butoxyethoxy)ethanol, during indoors application of paint by brushing over of periods of 57-149 minutes (Gijsbers et al, 2004). The measurements were made using cotton sampling gloves which were worn over new protective gloves, where present. The amount of used product was between 0.5 and 2.5 litres and the paint contained between 0.4 and 3.2 % DEGBE. The treated area during measurements was between 2 and 15 m². Exposure was mainly due to exposure on the hands. The geometric means for potential hand exposure were 555 (range 4-18269) mg for filling (n = 30), 217 (range 0.3-27745) mg for loading (n = 28), and 98 (range 11-733) mg for brushing (n = 24). The estimated arithmetic and geometric means for the exposure rate were 0.091 and 0.045 (GSD 3.6) µg/cm²/min, respectively, assuming a hand surface area of 820 cm².

Conduct initial risk characterisation (3.2.6):

As indicated above, a health-based reference value for dermal exposure to 2-BE can be set at 30 mg/kg body weight. Although there are no direct measurements of dermal exposure to 2-BE available, an estimate was made using a DEGBE, an analogous but less volatile glycol ether (Gijsbers et al, 2004).

Assuming an average body weight of 70 kg, the EASE estimates of 21 to 420 mg/day would lead to dermal exposure of 0.3 and 6 mg/kg, indicating that the health risk due to dermal exposure

during formulating and handling of 2-BE containing paints was well controlled. Similarly, the reported geometric means of 555, 217 and 98 mg for filling, loading and brushing operations, respectively, would give corresponding dermal exposure estimates of 7.9, 3.1 and 1.4 mg/kg. Although these values are well below the health-based reference value, the risk characterisation is not very satisfying as the reported ranges for the study with DEGBE suggest that in the extreme cases the dermal exposure might be an order of magnitude higher than the reference value. This is also reflected in the much higher exposure estimates obtained by the RISKOFDERM model.

Refined risk characterisation (according to 3.3 and Figure 3)

Conduct bio-monitoring study (3.3.8):

Since 2-BE is readily absorbed through the skin and it was recognised that dermal exposure is likely, biological monitoring methods were developed, measuring the main urinary metabolite of 2-BE, 2-butoxyacetic acid (2-BAA) (Angerer et al, 1990; NIOSH, 1990; Söhnlein et al, 1993; Vincent et al, 1996; Haufroid et al, 1997; Laitinen, 1998; INSERM, 1999). The correlation between exposure to 2-BE and the urinary excretion of 2-BAA has been well established and several authorities have set biological exposure limits for urinary 2-BAA (Boogaard, 2009). An eight-hour inhalation exposure to 5 ppm, in absence of dermal exposure, corresponds to a 2-BAA excretion of about 75 mmol/mol creatinine (88 mg/g creatinine) in post-shift urine samples. All current biological exposure limits are in this range.

If biological monitoring data are available with concomitant personal air monitoring data, one can calculate the expected urinary level of 2-BAA from the measured airborne concentrations of 2-BE using the known correlation between airborne exposure to 2-BE and the urinary excretion of BAA. If significant dermal exposure has occurred, the urinary excretion of 2-BAA will be larger than expected and the difference can be attributed to dermal exposure. In addition, extensive toxicokinetic data from human volunteer studies with neat 2-BE and aqueous solutions of 2-BE (Jakasa et al, 2004) and also a well-validated PBPK model are available (Corley et al, 1994; 1997; Franks et al, 2006). These also allow relating the 'excess' of BAA excretion, attributable to actually dermally absorbed 2-BE, to external dermal exposure to 2-BE. Application of this approach to the data reported by Söhnlein and co-workers (1993), using the most conservative correlation between exposure and urinary excretion (INSERM, 1999), the highest measured exposure and 30% absorption (Jakasa et al, 2004), the reasonable worst-case scenario for external exposure to 2-BE during formulation of product was calculated to be 278 mg/day (Boogaard, 2008). This value falls within the range estimated using EASE but shows a rather large discrepancy with the RISKOFDERM estimates. The most likely explanation is that the RISKOFDERM estimates are based on read-across to DEGBE which is 37 times less volatile than 2-BE. Due to the much slower evaporation of DEGBE

from the skin compared to 2-BE, more DEGBE is retained on the skin and available for absorption than 2-BE with equal initial deposition.

In conclusion, the refined health risk characterisation from dermal exposure to 2-BE using biological monitoring, indicates that the external exposure to 2-BE during formulation of product is approximately 300 mg/day or 4 mg/kg, assuming a body weight of 70 kg. This value confirms the EASE estimate and suggests there is no health risk associated with dermal exposure in this scenario.

GLOSSARY³

Benchmark dose (BMD): The statistical lower confidence limit of the dose corresponding to a small increase in effect over the background level. Typically, a 1% or 10% response level above the background is selected.

Biomonitoring: The measurement and assessment of workplace substances or their metabolites either in tissues, secretions, excretions or any combination of these to evaluate exposure and health risk compared to an appropriate reference.

Derived no effect level (DNEL): An exposure level above which humans should not be exposed.

Dermal exposure: Process of contact between an agent and human skin at a dermal exposure surface over an exposure period (quoted from CEN/TR 15278:2006).

Dose: Total amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population.

Dose-response relationship: Relationship between the amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the change developed in that organism, system or (sub)population in reaction to the agent.

Exposure: Concentration or amount of a particular agent that reaches a target organism, system, or (sub) population in a specific frequency for a defined duration.

Exposure assessment: Evaluation of the exposure of an organism, system, or (sub)population to an agent (and its derivatives).

Hazard: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub) population is exposed to that agent.

Hazard assessment: A process designed to determine the possible adverse effects of an agent or situation to which an organism, system or (sub)population could be exposed.

Health-based reference value: Maximum exposure value based on toxicological studies and appropriate assessment factors to protect humans from adverse health effects.

³ These definitions were mainly taken from WHO-IPCS, 2004 and WHO-IPCS, 2006.

Local effects: Adverse effect at the site of first contact (e.g. skin, eye, mucous membrane/gastro-intestinal tract, or mucous membrane/respiratory tract).

Margin of exposure: The ratio between a defined point on the dose-response curve for the adverse effect and the (human) intake.

No (observed) effect level (NOEL): The highest level of a test substance, to which organisms are exposed, that does not cause any observed and statistically significant adverse effects on the organism compared with the controls. For example, the NOEL might be the highest tested level at which an observed variable, such as growth, did not differ significantly from growth in the control. The NOEL customarily refers to sub-lethal effects and to the most sensitive effect unless otherwise specified.

Penetration: Entry of a substance into a particular layer or structure such as the entrance of a compound into the *stratum corneum*.

Percutaneous / dermal absorption: The process by which a substance is transported across the skin and taken up into the living tissue of the body.

Permeation: Penetration through one layer into another, which is both functionally and structurally different from the first layer.

Preparation: Means a mixture or solution composed of two or more substances (quoted from REACH, chapter 2, definitions and general provision, article 3. definitions; EU, 2006).

Resorption: Uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

Risk: The probability and severity of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent.

Risk assessment: A process intended to calculate or estimate the risk to a given target organism, system, or (sub) population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: Hazard identification, hazard characterisation, exposure assessment, and risk characterisation.

Risk characterisation: The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)population, under defined exposure conditions.

Risk characterisation ratio: Index that compares exposure concentration with critical effect (no-effect) concentration.

Risk management: Decision-making process involving considerations of political, social, economic, and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard. Risk management comprises three elements: Risk evaluation; emission and exposure control; and risk monitoring.

Stratum corneum: The outermost layer of the epidermis which consists of several layers of non-viable cells (typically 15-20). This layer varies in thickness with anatomical site and presents the major barrier to dermal absorption.

Substance: means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition (quoted from REACH, chapter 2, definitions and general provision, article 3. definitions; EU, 2006).

Systemic dose: The amount of a specified substance and/or its metabolites to which organs and tissues are exposed following absorption.

Systemic effect: A toxicological effect that affects the entire body or many organs.

Systemic exposure: Exposure of organs and tissues that occurs following absorption and distribution of a chemical in the body.

ABBREVIATIONS

2-BAA	2-Butoxyacetic acid
2-BE	2-Butoxyethanol
ACN	Acetonitrile
ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism, excretion
ATSDR	Agency for Toxic Substances and Disease Registry
BAA	Butoxyacetic acid
BAT	Biological tolerance value for occupational exposure
BEI	Biological exposure indice
BfR	Bundesinstitut für Risikobewertung / Federal Institute for Risk Assessment
BMD	Benchmark dose
CLP	Classification, Labelling and Packaging of substances and mixtures
COSHH	Control of substances hazardous to health
CSR	Chemical safety report
DEGBE	Diethylene glycol monobutyl ether
DG	Directorate General
DMEL	Derived minimum effect level
DNEL	Derived no effect level
DSD	Dangerous Substances Directive
EASE	Estimation and Assessment of Substance Exposure
ECB	(Former) European Chemicals Bureau
ECHA	European Chemicals Agency
ECPA	European Crop Protection Association
ECVAM	European Centre for the Validation of Alternative Methods
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
ESIS	European Chemical Substances Information System
EU	European Union
GHS	Globally harmonised system
GSD	Geometric standard deviation
HPV	High production volume
HSE	Health and Safety Executive

IARC	International Agency for Research on Cancer
IEH	Institute of environment and Health (UK)
INSERM	Institut National de la Santé et de la Recherche Médicale / National Health and Medical Research Institute
IPCS	International Programme on Chemical Safety
IPM	Isopropyl myristate
IRIS	Integrated Risk Information System (US EPA)
IUCLID	International Uniform Chemical Identification Database
IVC	Intake valve closure
JRC	Joint Research Centre
Kp	Permeability coefficient
LC	Lethal concentration
LD	Lethal dose
LLNA	Lode nymph node assay
LO(A)EL	Lowest observed (adverse) effect level
LOEL	Lowest observed effect level
LRI	Long-range research initiative (of Cefic)
MDA	Methylenedianiline
MoE	Margin of exposure
MRL	Maximum residue level
MTD	Maximum tolerated dose
MW	Molecular weight
NIOSH	National Institute for Occupational Safety and Health
NO(A)EL	No observed (adverse) effect level
NOEL	No observed effect level
NTP	US National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational exposure limit
OSHA	Occupational Safety and Health Administration
PBPK	Physiologically-based pharmacokinetic
PTFE	Polytetrafluoroethylene
QSAR	Quantitative structure-activity relationship
QSPR	Quantitative structure-permeation relationship

RCR	Risk characterisation ratio	
REACH	Registration, evaluation, authorisation and restriction of chemicals	
RISKOFDERM	Risk assessment of occupational dermal exposure to chemicals	
RMM	Risk management measures	
RPE	Respiratory protection	
SAR	Structure activity relationship	
SAT	Skin absorption time	
SCCS	Scientific Committee on Consumer Safety	
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks	Risks
SCHER	Scientific Committee on Health and Environmental Risks	
SIDS	Screening information data set (OECD)	
TCSA	Toxic Substances Control Act	
TGD	Technical Guidance Document	
TLV	Threshold limit value	
TRA	Targeted risk assessment	
TTC	Threshold of toxicological concern	
TWA	Time-weighted average	
US EPA	United States Environmental Protection Agency	
WHO	World Health Organisation	

BIBLIOGRAPHY

ACGIH. 2007. Threshold limit values and biological exposure indices. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, USA.

Angerer J, Lichterbeck E, Begerow J, Jekel S, Lehnert G. 1990. Occupational chronic exposure to organic solvents. XIII. Glycoether exposure during the production of varnishes. *Int Arch Occup Environ Health* 62: 123-126.

Anissimov YG, Jepps OG, Dancik Y, Roberts MS. 2013. Mathematical and pharmacokinetic modelling of epidermal and dermal transport processes. *Adv Drug Deliv Rev* 65(2):169-190.

BfR. 2010. Establishment of assessment and decision criteria in human health risk assessment for substances with endocrine disrupting properties under the EU plant protection product regulation. Report of a Workshop hosted at the German Federal Institute for Risk Assessment (BfR). Berlin, Germany, 15 January 2010.

Boogaard PJ. 2008. Biomonitoring as a tool in the human health risk characterization of dermal exposure. *Hum Exp Toxicol* 27(4):297-305.

Boogaard PJ. 2009. Biomonitoring of the Workplace and Environment (Text book). In Ballentyne B, Marrs T, Syversen T, eds, *General and Applied Toxicology*, 3rd Edition. Wiley, Chichester, UK, pp 2559-2589.

Boogaard PJ, Denneman MA, Sittert NJV. 2000. Dermal penetration and metabolism of five glycidyl ethers in human, rat and mouse skin. *Xenobiotica* 30:469-483.

Boogaard PJ, Hays SM, Aylward LL. 2011. Human biomonitoring as a pragmatic tool for health risk management of chemicals – Examples under the EU REACH Programme. *Regul Toxicol Pharmacol* 59:125-132.

Bos PMJ, Brouwer DH, Stevenson H, Boogaard PJ, de Kort WLAM, Van Hemmen JJ. 1998. Proposal for the assessment of quantitative dermal exposure limits in occupational environments: Part 1. Development of a concept to derive a quantitative dermal occupational exposure limit. *Occup Environ Med* 55(12):795–804.

Bouwman T, Cronin MT, Bessems JG, van de Sandt JJ. 2008. Improving the applicability of (Q)SARs for percutaneous penetration in regulatory risk assessment. *Hum Exp Toxicol* 27(4):269-276.

Brouwer DH, Hoogendoorn L, Bos PMJ, Boogaard PJ, Van Hemmen JJ. 1998. Proposal for the assessment of quantitative dermal exposure limits in occupational environments: Part 2. Feasibility study for application in an exposure scenario for MDA by two different dermal exposure sampling methods. *Occup Environ Med* 55(12):805-811.

Brouwer DH, Semple S, Marquart J, Cherrie JW. 2001. A dermal model for spray painters. Part I: Subjective exposure modelling of spray paint deposition. *Ann Occup Hyg* 45(1):15-23.

Capt A, Luzy AP, Esdaile D, Blanck O. 2007. Comparison of the human skin grafted onto nude mouse model with *in vivo* and *in vitro* models in the prediction of percutaneous penetration of three lipophilic pesticides. *Regul Toxicol Pharmacol* 47(3):274-287.

Cheng YS, Chen BT, Yeh HC. 1986. Size measurement of liquid aerosols. *J Aerosol Sci* 17:803-809.

Cleek RL, Bunge AL. 1993. A new method for estimating dermal absorption from chemical exposure. 1. General Approach. *Pharm Res* 10:497-506.

Corley RA, Bormett GA, Ghanayem BI. 1994. Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans. *Toxicol Appl Pharmacol* 129:61-79.

Corley RA, Markham DA, Banks C, Delorme P, Masterman A, Houle JM. 1997. Physiologically based pharmacokinetics and the dermal absorption of 2-butoxyethanol vapor by humans. *Fundam Appl Toxicol* 39:120-130.

Cronin MTD, Worth AP. 2008. (Q)SARs for predicting effects relating to reproductive toxicity. *QSAR Comb Sci* 27(1):91-100.

Cronin MT, Jaworska JS, Walker JD, Comber MH, Watts CD, Worth AP. 2003. Use of QSARs in international decision-making frameworks to predict health effects of chemical substances. *Environ Health Perspect* 111:1391-1401.

Cruz-Monteagudo M, Cordeiro MN, Borges F. 2008. Computational chemistry approach for the early detection of drug-induced idiosyncratic liver toxicity. *J Comput Chem* 29(4):533-549.

Dancik Y, Miller MA, Jaworska J, Kasting GB. 2013. Design and performance of a spreadsheet-based model for estimating bioavailability of chemicals from dermal exposure. *Adv Drug Deliv Rev* 65(2):221-236.

DuBois D, DuBois DF. 1916. A formula to estimate the approximate surface area if height and weight be known. *Arch Int Med* 17:863-871.

Durrheim H, Flynn GL, Higuchi WI, Behl CR. 1980. Permeation of hairless mouse skin: Experimental methods and comparison with human epidermal permeation by alkanols. *J Pharm Sci* 69(7):781-786.

EC. 1993. Council Directive 93/21/EEC. Approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Communities Vol L 110*, 04/05/1993.

EC. 2000. Commission Directive 2000/39/EC of 8 June 2000 establishing a first list of indicative occupational limit values in implementation of Council Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work. *Official Journal of the European Communities Vol L 142*.

EC. 2001. European Union Risk Assessment Report: 4,4'-methylene dianiline. European Communities. Luxembourg.

EC. 2004. Guidance document on dermal absorption. European Commission Health and Consumer Protection Directorate General Sanco 222/2000 rev7.

EC. 2008. European Union Risk Assessment Report: 2-butoxyethanol. Part 2 – Human health. European Communities, Luxembourg.

ECETOC. 1990a. Skin sensitisation testing. Monograph No. 14. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1990b. Skin irritation. Monograph No. 15. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1993a. Strategy for assessing a skin notation. Document No. 31. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1993b. Percutaneous absorption. Monograph No. 20. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium, pp 1-80.

ECETOC. 1995. Skin irritation and corrosion: Reference chemicals data bank. Technical Report No. 66. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1999a. Skin and respiratory sensitisers: Reference chemicals data bank. Technical Report No. 77. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 1999b. Skin sensitisation testing: Methodological considerations. Technical Report No. 78. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2000. Skin sensitisation testing for the purpose of hazard identification and risk assessment. Monograph No. 29. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2002. Use of human data in hazard classification for irritation and sensitisation. Monograph No. 32. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2003. Contact sensitisation: Classification according to potency. Technical Report No. 87. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2008. Potency values from the local lymph node assay: Application to classification, labelling and risk assessment. Document No. 46. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2010. Guidance on assessment factors to derive a DNEL. Technical Report No.110. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECHA REACH TGD. Technical Guidance Documents for the implementation of REACH. Guidance on information requirements and chemical safety assessment. European Chemicals Agency, Helsinki, Finland. (<http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>)

ECHA. 2009a. Practical Guide 6: How to report read-across and categories. European Chemicals Agency, Helsinki, Finland.

ECHA. 2009b. Practical Guide 5: How to report (Q)SARs. European Chemicals Agency, Helsinki, Finland.

ECHA. 2010. Opinion on new scientific evidence on the use of boric acid and borates in photographic applications by consumers. ECHA/RAC/A77-O-0000001273-82-05/F. ECHA Committee for Risk Assessment, April 29, 2010.

ECPA. 2010. Defining 'negligible exposure' within the context of Regulation 1107/2009, European Crop Protection Association. Document PP/10/EJ2013. 19 October 2010.

EEC. 1967. Council Directive 67/548/EEC (as amended for the seventh time by the Directive 92/32/EEC) on the approximation of the laws, regulations, administrative procedures relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities Vol L 196, 16/08/1967.

EFSA. 2012a. Scientific opinion on exploring options for providing advice about possible human health risks based on the concept of threshold of toxicological concern (TTC). European Food Safety Agency. EFSA Journal 10(7):2750.

EFSA. 2012b. Guidance on Dermal Absorption. European Food Safety Authority Panel on Plant Protection Products and their Residues (PPR). EFSA Journal 10(4):2665-2695. doi:10.2903/j.efsa.2012.2665.

EU. 1993. Council Regulation 793/93 on the evaluation and control of the risks of existing substances. OJL084, 5th April 1993.

EU. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Communities Vol L 396, 30/12/2006.

EU. 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Communities Vol L 353, 31/12/2008.

EU. 2009. Regulation (EC) No 1107/2009 of The European Parliament and of The Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Fasano WJ, McDougal JN. 2008. *In vitro* dermal absorption rate testing of certain chemicals of interest to the occupational safety and health administration: Summary and evaluation of US EPA's mandated testing. Regul Toxicol Pharmacol 51(2):181-194.

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, Henry CJ, eds, *Principles of route-to-route extrapolation for risk assessment*. Elsevier, New York, NY, USA, pp 93–127.

Franks SJ, Spendiff MK, Cocker J, Loizou GD. 2006. Physiologically based pharmacokinetic modelling of human exposure to 2-butoxyethanol. *Toxicol Lett* 162(2-3):164-173.

Gallegos Saliner A, Patlewicz G, Worth AP. 2005. A similarity based approach for chemical category classification. EUR 21867 EN. European Commission, Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau, Ispra, Italy.
(ecb.jrc.it/documents/QSAR/Report_Chemical_Similarity_for_Category_Classification.pdf)

Geinoz S, Guy R, Testa B, Carrupt PA. 2004. Quantitative structure-permeation relationships (GSPeRs) to predict skin permeation: a critical evaluation. *Pharm Res* 21:83-92.

Gijsbers JHJ, Tielemans E, Brouwer DH, Van Hemmen JJ. 2004. Dermal exposure during filling, loading and brushing with products containing 2-(2-butoxyethoxy)ethanol. *Ann Occup Hyg* 48:219-227.

Grégoire S, Ribaud C, Benech F, Meunier JR, Garrigues-Mazert A, Guy RH. 2009. Prediction of chemical absorption into and through the skin from cosmetic and dermatological formulations. *Br J Dermatol* 160(1):80-91.

Guerin T, Cummings L, Roser K. 2005. Hydroquinone monomethyl ether (4-methoxyphenol): Measurement of the *in vitro* rate of percutaneous absorption through human skin – Final report. Safety Assessment Group Health and Environment Laboratories (HAEL), Eastman Kodak Company, Rochester, New York, USA.

Hamey PY. 2001. An example to illustrate the potential use of probabilistic modelling to estimate operator exposure to pesticides. *Ann Occup Hyg* 45(1001):S55-S64.

Haufroid V, Thirion F, Mertens P, Buchet JP, Lison D. 1997. Biological monitoring of workers exposed to low levels of 2-butoxyethanol. *Int Arch Occup Environ Health* 70:232-236.

Hennes EC. 2012. An overview of values for the threshold of toxicological concern. *Toxicol Lett* 211:296-303.

Idson B. 1975. Percutaneous absorption. *J Pharm Sci* 64(6):901-924.

Idson B, Behl CR. 1987. Drug structure vs. penetration. In Kydonieus AF, Berner B, eds, *Transdermal Delivery of Drugs, Vol III*. CRC Press, Boca Raton, FL, USA, pp 85-151.

IEH. 2006. Guidelines on route-to-route extrapolation of toxicity data when assessing health risk of chemicals. Interdepartmental Group on Health Risks from Chemicals, Institute of Environment and Health, UK. April 2006. (www.silsoe.cranfield.ac.uk/ieh/pdf/cr12.pdf)

INSERM. 1999. Ethers de glycol - Quels risques pour la santé? Chpt. 10 Surveillance biologique de l'exposition. INSERM, Paris, pp 263-270.

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

Jones AD, Dick IP, Cherrie JW, Cronin MTD, Van De Sandt JJM, Esdaile DJ, Iyengar S, ten Berge W, Wilkinson SC, Roper CS, Semple S, de Heer C, Williams FM. 2004. CEFIC workshop on methods to determine dermal penetration for human risk assessment. European Chemical Industry Council, December, pp 1-86 (Research Report TM/04/07). http://www.iom-world.org/pubs/IOM_TM0407.pdf.

Karadzovska D, Brooks JD, Monteiro-Riviere NA, Riviere JE. 2013. Predicting skin permeability from complex vehicles. *Adv Drug Deliv Rev* 65(2):265-277.

Keller D, Krauledat M, Scheel J. 2009. Feasibility study to support a threshold of sensitisation concern concept in risk assessment based on human data. *Arch Toxicol* 83(12):1049-1060.

Klimisch HJ, Andrae M, Tillman U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul Toxicol Pharmacol* 25:1-5.

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G. 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem Toxicol* 42:65-83.

Kroes R, Renwick AG, Feron V, Galli CL, Gibney M, Greim H, Guy RH, L'Huguenot JC, van de Sandt JJM. 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. *Food Chem Toxicol* 45:2533-2562.

Laitinen J. 1998. Correspondence between occupational exposure limit and biological action level values for alkoxyethanols and their acetates. *Int Arch Occup Environ Health* 71:117-124.

Liron Z, Cohen S. 1984. Percutaneous absorption of alkanolic acids. I. A study of operational conditions. *J Pharm Sci* 73(4):534-537.

Lushington GH, Guo JX, Hurley MM. 2006. Acetylcholinesterase: Molecular modeling with the whole toolkit. *Curr Top Med Chem* 6(1):57-73.

McCarley KD, Bunge AL. 2001. Pharmacokinetic models of dermal absorption. *J Pharm Sci* 90:1699-1719.

McDougal JN, Boeniger M. 2002. Methods for assessing risks of dermal exposures in the workplace. *Crit Rev Toxicol* 4:291-327.

McDougal JN, Jurgens-Whitehead JL. 2001. Short-term dermal absorption and penetration of chemicals from aqueous solutions: Theory and experiment. *Risk Anal* 21(4):719-726.

Mitragotri S, Anissimov YG, Bunge AL, Frasch HF, Guy RH, Hadgraft J, Kasting GB, Lane ME, Roberts MS. 2011. Mathematical models of skin permeability: an overview. *Int J Pharm* 418(1):115-129.

Moss GP, Dearden JC, Patel H, Cronin MTD. 2002. Quantitative structure-permeability relationships (QSPRs) for percutaneous absorption. *Toxicol in vitro* 16(3):299-317.

NIOSH. 1990. Criteria for a Recommended Standard. Occupational exposure to ethylene glycol monobutyl ether and ethylene glycol monobutyl ether acetate. National Institute for Occupational Safety and Health, US Dept of Health and Human Services, Public Health Service, Centers for Disease Control, Washington, DC, USA.

Northage C. 2005. EASEing into the future. *Ann Occup Hyg* 49(2):99-101.

OECD. 1997. OECD Guideline for the Testing of Chemicals. Test No. 471: Bacterial reverse mutation test. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2004a. OECD Guideline for the Testing of Chemicals. Test 430: *In vitro* skin corrosion: Transcutaneous electrical resistance test (TER). Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2004b. OECD Guideline for the Testing of Chemicals. Test 431: *In vitro* skin corrosion: Human skin model test. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2004c. OECD Guideline for the Testing of Chemicals. Test 428: Skin absorption: *In vitro* method. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2004d. Guidance document for the conduct of skin absorption studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28; ENV/JM/MONO(2004). Organisation for Economic Co-operation and Development, Environment Directorate, Paris, France, pp 1-31.

OECD. 2004e. OECD guideline for the testing of chemicals. Skin absorption: *In vivo* method. Test No. 427. Adopted: 13 April 2004. Organisation for Economic Co-operation and Development, Paris, France, pp 1-8.

OECD. 2005. Ch. 3.2 Guidance of the development and use of chemical categories in the HPV chemicals programme in Chapter 3 Data Evaluation in Manual for Investigation of HPV Chemicals. (www.oecd.org/document/7/0,2340,en_2649_201185_1947463_1_1_1_1,00.html)

OECD. 2009. OECD guideline for the testing of chemicals. Subacute inhalation toxicity: 28-day study. Test No. 412. Adopted: 7 September 2009. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2010. OECD Guideline for the Testing of Chemicals. Test No. 429: Skin Sensitisation: Local Lymph Node Assay. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2011. Guidance notes on dermal absorption. Series on Testing and Assessment, No. 156, 18 August 2011. ENV/JM/MONO(2011)36. Organisation for Economic Co-operation and Development, Paris, France.

Oppl R, Kalberlah F, Evans PG, van Hemmen JJ. 2003. A toolkit for dermal risk assessment and management: an overview. *Ann Occup Hyg* 47(8):629-640.

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

Ridout G, Guy RH. 1988. Structure-penetration relationships in percutaneous absorption. *ACS Symposium Series* 371:112-123.

Ridout G, Houk J, Guy RH, Santus GC, Hadgraft J, Hall LL. 1992. An evaluation of structure-penetration relationships in percutaneous absorption. *Il Farmaco* 47:869-892.

Rila JP, Bos PMJ, Hulzebos E, Hakkert BC. 2006. Workability of the guidance documents for the category or read-across approach for selected groups of chemicals. RIVM report 601200009/2006. Dutch National Institute of Public Health and the Environment. (www.rivm.nl/bibliotheek/rapporten/601200009.pdf)

Roberts MS, Walters KA. 1998. The relationship between structure and barrier function of skin. In Roberts MS, Walters KA, eds, *Dermal absorption and toxicity assessment*. Marcel Dekker, New York, NY, USA pp 1-42.

Rosemond ZA, Lladós F. 1998. Toxicological profile for methylenedianiline. ATSDR Tox Profiles. US Department of Health and Human Services, pp 1-158.

Safford RJ. 2008. The dermal sensitisation threshold – a TTC approach for allergic contact dermatitis. *Regul Toxicol Pharmacol* 51:195-200.

Safford RJ, Aptula AO, Gilmour N. 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. *Regul Toxicol Pharmacol* 60:218-224.

Sartorelli P, Aprea C, Cenni A, Novelli M, Orsi D, Palmi S, Matteucci G. 1998. Prediction of percutaneous absorption from physicochemical data: A model based on data for *in-vitro* experiments. *Ann Occup Hyg* 42:267-276.

SCCS, SCHER, SCCP, SCENIHR. 2012. Joint opinion on the use of the threshold of toxicological concern (TTC) approach for human safety assessment of chemical substances with focus on cosmetics and consumer products. SCCP/1171/08.

Scheuplein RJ, Blank IH. 1971. Permeability of the skin. *Physiol Rev* 51:702-747.

Schwindt DA, Wilhelm KP, Maibach HI. 1998. Water diffusion characteristics of human *stratum corneum* at different anatomical sites *in vivo*. *J Invest Dermatol* 111:385-389.

Semple S, Brouwer DH, Dick F, Cherrie JW. 2001. A dermal model for spray painters. Part II: Estimating the deposition and uptake of solvents. *Ann Occup Hyg* 45:25-33.

Söhnlein B, Letzel S, Weltle D, Rudiger HW, Angerer J. 1993. Occupational chronic exposure to organic solvents. XIV. Examinations concerning the evaluation of a limit value for 2-ethoxyethanol and 2-ethoxyethyl acetate and the genotoxic effects of these glycol ethers. *Int Arch Occup Environ Health* 64:479-484.

ten Berge WF. 2009. A simple dermal absorption model: derivation and application. *Chemosphere* 75:1440-1445.

Toxmatch Version 1.05 is available for download from the ECB website. For more details, please look under 'QSAR Tools' under the ECB QSAR webpage or click on the following link: (ecb.jrc.it/qsar/qsar-tools/index.php?c=TOXMATCH)

US EPA. 1992. Dermal Exposure Assessment: Principles and Applications, Interim Report; 1992, EPA/600/8-91/011B. United States Environmental Protection Agency, USA.

US EPA. 1998. Health Effects Test Guidelines: Dermal penetration. OPPTS 870.7600. United States Environmental Protection Agency, USA.

US EPA. 2004a. Toxicological review of boron and compounds (CAS No. 7440-42-8) in support of summary information on the Integrated Risk Information System (IRIS). United States Environmental Protection Agency, USA.

US EPA. 2004b. Risk assessment guidance for Superfund, Vol I: Human health evaluation manual (Part E, Supplemental guidance for dermal risk assessment) final. EPA/540/R/99/005 OSWER 9285.7-02EP PB99-9633124b. United States Environmental Protection Agency, USA.

US EPA. 2007. Dermal Exposure Assessment: A summary of EPA approaches. EPA/600/R-07/040F. United States Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC, USA.

Van Hemmen JJ. 2001. EUROPOEM, a predictive occupational exposure database for registration purposes of pesticides. *Appl Occup Environ Hyg* 16(2):246-250.

Van Hemmen JJ, Auffarth J, Evans PG, Rajan-Sithamparanadarajah B, Marquardt, H, Oppl R. 2003. Risk assessment of occupational dermal exposure to chemicals. An Introduction to a series of papers on the development of a toolkit. *Ann Occup Hyg* 47:595-598.

Van Ravenzwaay N, Leibold E. 2004a. A comparison between *in vitro* rat and human and *in vivo* rat skin absorption studies. *Human Exp Toxicol* 23:421-430.

Van Ravenzwaay N, Leibold E. 2004b. The significance of *in vitro* rat skin absorption studies to human risk assessment. *Toxicol in Vitro* 18:219-225.

Van Wendel de Joode B, Brouwer DH, Vermeulen R, Van Hemmen JJ, Heederik D, Kromhout H. 2003. DREAM: A method for semi-quantitative dermal exposure assessment. *Ann Occup Hyg* 47(1):71-87.

Van Wendel de Joode B, Vermeulen R, Van Hemmen JJ, Fransman W, Kromhout H. 2005. Accuracy for a semi-quantitative method for dermal exposure assessment (DREAM). *Occup Environ Med* 62:623-632.

Vecchia BE, Bunge AL. 2002a. Skin absorption databases and predictive equations. In Guy RH, Hadgraft J, eds, *Transdermal drug delivery*, Chapter 3. Marcel Dekker.

Vecchia BE, Bunge AL. 2002b. Partitioning of chemicals into skin: results and predictions. In Guy RH, Hadgraft J, eds, *Transdermal drug delivery*, Chapter 4. Marcel Dekker.

Vincent R, Rieger B, Subra I, Poirot P. 1996. Exposure assessment to glycol ethers by atmosphere and biological monitoring. *Occupational Hygiene* 2:79-99.

Walker JD, Dimitrov S, Mekenyan O. 2003. Using HPV chemical data to develop QSARs for non-HPV chemicals: opportunities to promote more efficient use of chemical testing resources. *QSAR Comb Sci* 22:386-395.

Wester RC, Maibach HI. 1985. *In vivo* percutaneous absorption and decontamination of pesticides in humans. *J Toxicol Environ Health* 16:25-37.

Wester RC, Hui X, Hartway T, Maibach HI, Bell K, Schell MJ, Northington DJ, Strong P, Culver BD. 1998. *In vivo* percutaneous absorption of boric acid, borax, and disodium octaborate tetrahydrate in humans compared to *in vitro* absorption in human skin from infinite and finite doses. *Toxicol Sci* 45(1):42-51.

WHO-IPCS. 2004. *IPCS Risk Assessment Terminology*. Harmonization Project Document No.1. International Programme on Chemical Safety. World Health Organisation, Geneva, Switzerland.

WHO-IPCS. 2006. *Dermal Absorption*. Environmental Health Criteria 235. International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland.

Wilschut A, ten Berge W F, Robinson P J, McKone T E. 1995. Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* 30:1275-1296. <http://home.planet.nl/~wtberge/qsarperm.html>

Wu S, Blackburn K, Amburgey J, Jaworska J, Federle T. 2010. A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. *Regul Toxicol Pharmacol* 56:67-81.

APPENDIX A: QSARS FOR SYSTEMIC HEALTH EFFECTS

There are limited QSARs for systemic effects, however there is a great potential in this area. As with all QSAR models these are restricted by the quality and quantity of the data available for modelling. In addition, toxicological information from case studies may be available for particular chemicals, but putting this information together to form a consistent database may not be possible. This does not imply that it is not possible to develop succinct and usable models merely they have seldom been developed and lack the fundamental information.

With regard to reproductive toxicity, Cronin and Worth (2008) reviewed the available QSARs. This is probably one of the most complex areas to derive models for, although a number of expert systems are available that include information relevant to endpoints that are associated with reproductive toxicity. A small number of structural alerts to identify modes and mechanisms of reproductive toxicants are available (e.g. in the DEREK for Windows software). More quantitative models have been developed for a variety of endpoints with data sets derived from limited and highly related series, or more heterogeneous series of compounds. Several expert systems (e.g. MCASE and TOPKAT) offer predictive capabilities for reproductive endpoints. Of the reproductive toxicity endpoints, the greatest progress has been in the area of endocrine disruption. These algorithms are commonly applied by expert systems such as OASIS.

There are few QSARs for target organ endpoints. However, there is a possibility for links to work in drug discovery, particularly where specific effects are of concern. In terms of modelling, there are few internationally accepted endpoints (e.g. with OECD guidelines) which makes creating databases for modelling more difficult.

There are more data, and consequently more models, in areas commonly addressed in the development of pharmaceuticals. There are a number of QSAR models for (idiosyncratic) liver toxicity (e.g. Cruz-Monteagudo et al, 2008). In addition there are modules with expert systems such as MCASE. It is probable that these models cross a variety of mechanisms, even if individual mechanisms are known. A significant problem with regard to REACH is that most, if not all, data for liver toxicity are for drug compounds which may fall into a separate part of the chemical universe from REACH chemicals. There are few models directly for neurotoxicity, as this is again a diverse endpoint. However, there is a very rich literature on related effects such as acetylcholinesterase inhibition (see the recent review by Lushington et al, 2006). Some of this could be applied in particular areas to assist in the screening of compounds that may affect nerve transmission.

The most successful application of (Q)SAR to predict systemic toxicity for risk assessment in the future is likely to be based around read-across approaches. There is a growing realisation that values such as the NOEL are of limited use for model building. Instead, future progress will be

the development of models, structural alerts and groupings for organ level toxicity. As an example, version 3.0 of the OECD QSAR Toolbox contains a new profiler for repeat-dose toxicity and new data. However, the current state of the art is that the currently available tools are, at best, only suitable for screening and prioritisation and will provide only limited use for risk assessment of complex toxicological endpoints such as systemic toxicity.

APPENDIX B: MODELLING APPROACH TO SOLVENT EXPOSURE SCENARIO

In the publication by Semple et al (2001), exposure modelling is described for workers in the spray painting industry. The model allows total exposure (skin loading and absorption) to solvents to be estimated. The concepts can be used in other industries where dermal exposure occurs.

Estimating both the deposition and the chemical uptake through the skin provides a biologically relevant evaluation of the hazard. The exposure model uses information relating to the nature of the hazardous material, the job task, the degree of control used in the process, the time and quantities of chemical used and other environmental factors that can be used to estimate the quantity of chemical deposited on a worker's skin, and from this, the amount of chemical actually absorbed through the skin and systemically available.

The first stage of the model determines the skin exposure area. The model divides the painter's skin into two parts: exposed skin and skin beneath the clothing. The skin surface area related to the individual's height and weight is determined using the following from DuBois and DuBois (1916):

$$\text{TOTAL}_{\text{SURF}} = \text{WEIGHT}^{0.425} \times \text{HEIGHT}^{0.725} \times 71.84 \quad [1]$$

WEIGHT is measured in kilograms (kg), HEIGHT is in centimetres (cm) and $\text{TOTAL}_{\text{SURF}}$ is in cm^2 .

The second stage of the model estimates the total volume of paint that could be deposited on the skin for a given work routine using previously published factors required to populate the model of Brouwer et al (2001).

$$V_{\text{TOT}} = V \times T(1-n_{\text{SPRAY}}) \times O^{-1} \times EV \times V_{\text{NT}} \times WV \times P \times 0.01n_{\text{SPRAY}} \times D \quad [2]$$

V_{TOT} is the volume of paint deposited on painter (ml).

V is the volume rate of spraying (ml/min).

T is the spraying time (min).

n_{SPRAY} is a technique-related deposition efficiency factor.

V_{NT} is a ventilation factor.

WV is a worker's orientation to the air stream factor.

O is an object factor.

D is the distance factor.

P is a posture factor.

EV is an evaporation factor.

The third stage in the model estimates the total coverage area for the estimated volume of paint, both clothing and bare skin. In this example, this is done by converting the deposited volume into

the number of paint droplets impacting on the painter. Using experimental data (Cheng et al, 1986) the model then estimates the area covered by each impacting paint spray droplet. The physical area of clothing or skin that is covered by paint droplets over the paint spraying time is then a simple function of the number of droplets depositing on the painter and the size of the area that each droplet will cover; OECD estimates that each 10 l (a finite dose) will cover approximately 1 cm² of area without runoff.

Stage four of the model uses the thickness of the paint layer formed by a single droplet, together with the paint density and solvent content of the paint to calculate how long the paint will take to dry. This time reflects the period when solvent is available for uptake through the skin. The drying time is linked to the evaporation rate of the solvent which will in turn be influenced by surface temperature.

Stage five of the model uses a formula derived from recent work into the prediction of percutaneous absorption by Sartorelli et al (1998). This technique employs physiochemical data such as solubility in water and the octanol/water partition coefficient to estimate the probable rate of permeation of the substance through the skin:

$$K_p = 0.01498 + (0.00109 \times wt_{sol}) - (0.00113 \times \ln(K_{ow})) \quad [3]$$

wt_{sol} is the solubility of solvent in water (g/l).

K_{ow} is the octanol/water coefficient of solvent.

This results in the permeability coefficient of the solvent (K_p) and is measured in units of cm/h.

The rate of solvent flux through the skin (J) is the product of the permeability coefficient of the solvent and the skin surface concentration:

$$J = K_p \cdot C \quad [4]$$

Equation [4] predicts the mass of solvent that will pass through each exposed square centimetre of skin per hour.

Stage six of the model calculates the uptake of solvent during the paint drying time. Here it is assumed that all commonly used paint types will dry on the skin before the end of the lag period and so solvent absorption will therefore only take place prior to saturation. The model thus employs first-order kinetics to determine the amount of solvent absorbed per unit of exposed area over the paint drying time (which equates to the exposure time).

Stage seven of the model combines the uptake [penetration] rate of solvent over the exposed time period with the total area exposed as calculated previously. There is also a correction to ensure that multiple paint layer coverage does not create false results by describing larger body surfaces than actually exist. The uptake of solvent from both the uncovered skin and clothed areas is then summed to give total dermal uptake expressed in milligrams.

Stage eight is a simple calculation of the amount of solvent that is inhaled by the painter in the given environmental conditions and with known or previously estimated airborne concentrations of solvent. The calculation also takes into account the respiratory rate and volume of each breath (combined to give the minute volume), and any respiratory protection (RPE) the painter may be wearing:

$$U_{\text{inh}} = (R_{\text{VOL}} \times T \times C_{\text{AIR}}) \div \text{RPE} \quad [5]$$

U_{inh} mass of solvent absorbed by inhalation (mg).

R_{VOL} minute volume of respiration (m^3/min).

C_{AIR} concentration of solvent in air (mg/m^3).

T exposure time (min).

RPE workplace protection factor of any respiratory protection worn.

The final stage allows direct comparison of the solvent taken up dermally with that received by inhalation. This allows to conclude that dermal exposure provides a given proportion of the total solvent body burden and to determine a MoE.

APPENDIX C: APPROACHES FOR THE ACQUISITION OF RELEVANT DATA

Estimating internal dose using Fick's Law

The flux or the amount of chemical that penetrates a defined area of skin per unit of time is described by Fick's Law:

$$J = P \Delta C \quad [1]$$

Where J is flux or the transfer rate in units of mass/time/area, P is the permeability coefficient, or K_p in units of distance/time, and ΔC is the concentration difference across the skin membrane. When estimating skin penetration, it can be assumed that the skin is a simple homogenous membrane and that the chemical concentration on the surface skin is proportional to its flux and that movement of chemical through the skin is passive. Under steady-state penetration conditions, that is the dose to the skin is infinite and sink conditions are maintained through the skin and into the receptor fluid of an *in vitro* dermal diffusion cell model, flux is dependent on the thermodynamic activity of chemical and is specific for a chemical, a vehicle and a given species.

The amount of chemical that penetrates the skin barrier is directly proportional to the permeability of the chemical, the exposed surface area, the concentration and the exposure time. For skin exposure to chemicals in the form of liquids or mixtures, an internal dose (or body burden) can be estimated using the following equation (McDougal and Boeniger, 2002):

$$M = K_p \times A \times C \times t \quad [2]$$

Where M is the mass or internal dose absorbed (mg), K_p the permeability coefficient (cm/hour) and is determined either empirically or by correlation using quantitative structure permeation relationship models (QSPR), A is the skin surface area (cm²), C is the concentration of the chemical on the skin surface (mg/cm³) and t is the exposure time (hours).

K_p is most useful for quantifying the penetration of chemicals at various concentrations from a water vehicle (Flynn, 1990). Theoretically, K_p is concentration independent and chemical-vehicle specific.

For pure chemicals, either liquids or solids, because the thermodynamic behaviour is equivalent, flux is (theoretically) equivalent. Skin doses to solids or powders will increase with an increase in skin loading until a maximum flux is achieved (J_{max}). Under infinite skin loading conditions:

$$M = J_{\max} \times A \times t \quad [3]$$

Where M is the mass or internal dose absorbed (mg), J_{\max} is maximum flux under infinite skin loading (mg/cm²/hour), A is the exposure area (cm²), and t is the exposure time (hours).

Both equations [2] and [3] assume that the chemical's flux at steady-state is linear over the entire exposure interval. However, for short-term exposures, particularly those that are less than the lag time (a phenomenon of the *in vitro* diffusion cell model, the x-axis intercept, back-extrapolated from the linear portion of the best-fit line at steady-state), use of equations [2] and [3] may underestimate the amount absorbed *in vivo* since they do not account for chemical that is contained in skin, which may be significant for highly lipophilic chemicals.

Alternative to these equations for estimating absorbed dose, Cleek and Bunge (1993) with additional interpretation by McDougal and Jurgens-Whitehead (2001) proposed the following (the square root of time calculation), which accounts for not only chemical that has penetrated through the skin but also for chemical in the skin (prior to steady-state):

$$M = AC \sqrt{\frac{4(R\delta)Kp \times t}{\pi}} \quad [4]$$

Where M is the mass or internal dose absorbed (mg), A is the skin surface area (cm²), C is the concentration of the chemical on the skin surface (mg/cm³), R is the chemical's *stratum corneum*/water partition coefficient or K_m (unitless) and δ is the thickness of the *stratum corneum* (cm). The *stratum corneum*/water partition coefficient (R) can be approximated by the following function (McCarley and Bunge, 2001):

$$\log(K_{sc/w}) = a + b \cdot \log(K_{w/o}) \quad [5]$$

Where $K_{sc/w}$ is the *stratum corneum*/water partition coefficient, a and b are constants and $K_{o/w}$ is the water/octanol partition coefficient; $[a]$ and $[b]$ have been reported to be '0', and 0.74, respectively (Cleek and Bunge, 1993). The *stratum corneum* thickness (δ) for human skin (mean data) has been reported by Schwindt et al (1998) to range from 7.7 μm (abdomen) to 13.1 μm (thigh). The authors suggest that utility of equation [4] should be relegated to dermal exposures that are less than 2.4 times the lag time.

Alternatives to estimates of internal dose that adhere to Fick's Law are empirical, scenario-specific measurements to estimate the fractional portion of a chemical that is absorbed (usually reported as 'percent' absorbed). This is specific to the chemical concentration (skin loading) and exposure time. However, fractional absorption experiments have limited applicability across various chemical concentrations and exposure times. Nonetheless, use of specific formulations with a single concentration, skin loading and exposure time, typical within the agricultural

industry to predict absorbed doses for mixer/loaders and backpack spraying operations, produce a less conservative estimate of the absorbed dose than would be produced by equations 2-4.

Estimating internal dose using fractional absorption

Alternatives to estimates of internal dose that adhere to Fick's Law are empirical measurements to estimate the fractional portion or per cent of a chemical that is absorbed. This is specific to the chemical concentration (skin loading) and exposure time. Fractional absorption for infinite and finite doses increases with time; with finite doses, more typical of workplace exposures, flux decreases with time as the amount of chemical on the skin is depleted. The use of per cent absorbed or fractional absorption is not an accurate means of predicting dermal absorption for various concentrations or exposure times. The per cent absorbed value does not extrapolate accurately to other exposure scenarios.

Using defined conditions, a finite dose or monolayer on the skin, the utility of the fractional absorption approach is self-limiting as it prevents extrapolation to other time-dependent exposures; fractional absorption (scenario-specific conditions of dose and time) has limited application to the broader ability to predict an internal dose following dermal exposure to chemicals. However, in those industries where specific exposure scenarios are well defined, that is skin dose and exposure time are well understood (such is the case for crop protection chemicals or cosmetic applications) the weakness on a broader scale is actual a strength under these conditions. Nonetheless, flux of finite doses from chemicals may provide a reasonable model for understanding penetration and absorption from single and repeat finite exposures to chemicals over the course of a workday.

Example 1: Determining the skin absorption time to the TLV using Kp

Chemical:	4-methoxyphenol
CAS no.:	150-76-5
Molecular weight:	124.14 g/mol
TLV (ACGIH, 2007):	5 mg/m ³
ACGIH skin notation	No
Concentration (in water vehicle):	35 mg/ml
Kp (Guerin et al, 2005):	9.39 x 10 ⁻³ cm/hour
Skin exposure area (both hands):	840 cm ²

A. Calculate absorbed dose inhaled at the TLV:

$$\text{Amount absorbed at TLV (mg)} = \text{TLV (mg/m}^3\text{)} \times \text{8-hour inhalation (10 m}^3\text{)}$$

$$\text{Amount absorbed at TLV (mg)} = 5 \text{ mg/m}^3 \times 10 \text{ m}^3$$

$$\text{Amount absorbed at TLV} = 50 \text{ mg}$$

B. Determine the skin absorption time (SAT) for exposure to hands:

$$\text{SAT} = \text{total absorption at TLV or regulatory level (8 hours/workday)} \div (\text{Kp} \times \text{concentration} \times \text{skin area})$$

$$\text{SAT} = 50 \text{ mg} \div (9.39 \times 10^{-3} \times 35 \text{ mg/mL} \times 840 \text{ cm}^2)$$

$$\text{SAT} = 0.18 \text{ hours}$$

Based on the measured Kp for 4-methoxyphenol from an aqueous vehicle, and assuming continuous exposure to the hands, in absence of any body burden from inhalation exposure at the TLV, the SAT is estimated to be 0.18 hours or 11 minutes.

Example 2: Determining the skin absorption time to the TLV for a neat chemical using steady-state flux

Chemical:	Acetonitrile
CASN:	75-05-8
Molecular weight:	41.05 g/mol
TLV (ACGIH, 2007):	33.6 mg/m ³
ACGIH skin notation	Yes
Density (neat):	787 mg/ml
Infinite dose flux (Fasano and McDougal, 2008):	0.143 mg/cm ² /hour
Skin exposure area (both hands):	840 cm ²

A. Calculate absorbed dose inhaled at the TLV:

$$\text{Amount absorbed at TLV (mg)} = \text{TLV (mg/m}^3\text{)} \times \text{8-hour inhalation (10 m}^3\text{)}$$

$$\text{Amount absorbed at TLV (mg)} = 33.6 \text{ mg/m}^3 \times 10 \text{ m}^3$$

$$\text{Amount absorbed at TLV} = 336 \text{ mg}$$

B. Determine the SAT for exposure to hands:

$$SAT = \text{total absorption at TLV or regulatory level (8 hours/workday)} \div (\text{flux} \times \text{skin area})$$

$$SAT = 336 \text{ mg} \div (0.143 \text{ mg/cm}^2/\text{hour} \times 840 \text{ cm}^2)$$

$$SAT = 2.8 \text{ hours}$$

Based on the steady-state flux for acetonitrile applied neat under infinite dose conditions, and assuming continuous exposure to the hands, in absence of any body burden from inhalation exposure at the TLV, the SAT is estimated to be 2.8 hours.

APPENDIX D: QSARS FOR SKIN PERMEABILITY

QSARs for skin permeability have been reviewed by Moss et al (2002) and Walker et al (2003). The passage of chemicals across the skin may be viewed as a passive diffusion process. As such, most success from modelling skin permeability has come from the use of descriptors for hydrophobicity and molecular size. Also, a number of issues regarding data quality from historical sources have made modelling more complex. Assessing the ability of a chemical to cross the skin is important for risk assessment but need not necessarily be considered as a toxicity test (Cronin et al, 2003). There are a variety of *in vitro* and *in vivo* methodologies to assess percutaneous absorption. Probably the most widespread and potentially useful one is the use of excised human skin *in vitro*. Walker et al (2003) described the regulatory application of QSARs to predict dermal absorption of compounds. The permeability coefficient was predicted by a series of simple QSARs that were based either on hydrophobicity and molecular size or on hydrophobicity alone.

QSARs, when applied to estimating dermal permeability coefficients, are sometimes known as quantitative structure–permeability relationships (QSPeR). Recent overviews of QSPeRs for permeation into human skin from water are given by Moss et al (2002), Vecchia and Bunge (2002a,b), Walker et al (2003), Fitzpatrick et al (2004), and Geinoz et al (2004).

A QSAR provides predictions of coefficients needed to estimate absorption for untested chemicals. Mathematical models simulate the sequence of partition and transport processes involved in dermal chemical permeation through the skin (Fitzpatrick et al, 2004). Mathematical modelling plays a key role in linking permeability coefficient data obtained from tests under steady-state conditions (i.e. infinite dose) to absorption estimates for finite dose applications that are more typical of occupational exposure (i.e. non-steady-state conditions) (Jones et al, 2004).

QSPeRs often are derived using parameters that themselves are calculated from QSARs. For example, the octanol/water partition coefficient K_{ow} has often been used as an input parameter to QSPeR equations. While K_{ow} has been measured for some chemicals, for others a QSAR prediction is used, and these values will reflect the predictive limitations of the QSAR. Various software programs can provide differing estimates for K_{ow} and solute solubility.

Algorithms for the prediction of K_p , provided by Flynn (1990) and Potts and Guy (1992), demonstrated the use of $\log K_{ow}$ in combination with either molecular weight (MW) or molecular volume to predict the K_p data (in units of cm/hour) collected by Flynn (1990):

$$\text{Log } K_p = -2.74 + 0.71 \log K_{ow} - 0.0061 \text{ MW } (n = 93; r^2 = 0.67)$$

where n is the number of observations or number of compounds and r is the correlation coefficient.

Reviews of modelling the skin permeability of homologous, or closely related, compounds are given by Scheuplein and Blank (1971), Idson (1975), Wester and Maibach (1985), Idson and Behl (1987), Ridout and Guy (1988), and Ridout et al (1992).

US EPA (1992) and US EPA (2004b) presented a slightly modified version of the Potts and Guy (1992) correlation, which included an adjustment for the transport resistance through the viable epidermis, as proposed by Cleek and Bunge (1993). US EPA (2004b) also presented the effective prediction domain for this correlation — i.e. the range of K_{ow} values and molecular weights for which this equation is valid.

$$\text{Log } K_p = -2.80 + 0.66 \log K_{ow} - 0.0056 \text{ MW } (r^2 = 0.66)$$

It should be noted that there are some chemicals that fall outside the ‘effective prediction domain’ for determining K_p , in particular those with a high molecular weight and high K_{ow} values (lipophilic compounds accumulating in the *stratum corneum*). For halogenated chemicals, the predicted K_p could be underestimated due to the lower ratio of molar volume related to molecular weight compared with those included in the Flynn (1990) data set.

Grégoire et al (2009) developed a preliminary predictive model, using algorithms recommended by the US EPA, to predict the mass of a chemical absorbed into and through the skin from a cosmetic or dermatological formulation. This model was developed using *in vitro* human skin absorption data for 36 chemicals, and predictions were attempted for two chemicals. Given the limited number of chemicals evaluated to date in this approach, currently for regulatory purposes only, it is best considered a promising research model that requires further demonstration of its predictive power for non-steady-state dermal dosing scenarios.

The following references are to be noted as they are recent reviews on the topic: Anissimov et al, 2013; Bouwman et al, 2008; Dancik et al, 2013; Karadzovska et al, 2013; Mitragotri et al, 2011.

APPENDIX E: GUIDELINES FOR DERMAL ABSORPTION AND PENETRATION STUDIES

Current general guidance for conducting dermal absorption studies is contained in OECD Guidelines 427 and 428, and Guidance 28. Additionally there are guidelines produced by individual authorities (e.g. US EPA, OPPTS 870.7600). Aligned with these, there are a number of other guidance documents intended to act as aids for the design, performance and interpretation of these studies.

OECD. 2004c. OECD guideline for the testing of chemicals. Skin absorption: *in vitro* method. Test No. 428. Adopted: 13 April 2004. Organisation for Economic Co-operation and Development, Paris, France, pp 1–8.

OECD. 2004d. Guidance document for the conduct of skin absorption studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28; ENV/JM/MONO(2004). Organisation for Economic Co-operation and Development, Environment Directorate, Paris, France, pp 1–31.

OECD. 2004e. OECD guideline for the testing of chemicals. Skin absorption: *in vivo* method. Test No. 427. Adopted: 13 April 2004. Organisation for Economic Co-operation and Development, Paris, France, pp 1–8.

OECD. 2011. Guidance Notes on Dermal Absorption. Series on Testing and Assessment, No. 156, 18 August 2011. ENV/JM/MONO(2011)36. Organisation for Economic Co-operation and Development, Paris, France.

ECETOC. 1993b. Percutaneous absorption. Monograph No. 20. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium, pp 1-80.

EFSA. 2012b. Guidance on Dermal Absorption. European Food Safety Authority Panel on Plant Protection Products and their Residues (PPR). EFSA Journal 10(4):2665-2695. doi:10.2903/j.efsa.2012.2665.

US EPA. 1998. Health Effects Test Guidelines: Dermal penetration. OPPTS 870.7600. US Environmental Protection Agency, USA.

US EPA. 2007. Dermal Exposure Assessment: A summary of EPA approaches. EPA/600/R-07/040F. US Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC, USA.

WHO-IPCS. 2006. Dermal Absorption. Environmental Health Criteria 235. International Programme on Chemical Safety, World Health Organisation, Geneva, Switzerland.

When estimating skin penetration, it can be assumed that the skin is a simple homogenous membrane, a chemical's concentration on the skin surface is proportional to its flux and movement through the skin is passive. Under steady-state penetration conditions, i.e. the dose to the skin is infinite and sink conditions are maintained through the skin and into the receptor fluid

of an *in vitro* dermal diffusion cell model, flux is dependent on the thermodynamic activity of and specific for a chemical, a vehicle and a given species.

Current testing guidelines for dermal absorption studies, a synthesis

a) OECD/EU/US EPA

This section is an attempt to combine the information given in the OECD guidelines, OECD guidance document and the EU guidance document for the conduct of *in vitro* and *in vivo* dermal absorption studies. The current EPA guideline for dermal absorption (OPPTS 870.7600) only deals with *in vivo* methods as *in vitro* data were not, until recently, considered in risk assessments. More recently *in vitro* methods have been accepted as supportive evidence along with an *in vivo* study. This acceptance is indicated in the document 'Dermal Exposure Assessment: A Summary of EPA Approaches' issued in 2007.

There is also a section entitled 'additional dermal absorption studies' in the OPPTS 870.7600 guideline that describes outlines for studies where significant quantities of residue remained on washed skin (similar to OECD guideline in that a depuration period for collecting excreta is suggested to investigate the fate of the material in/on the skin following washing), there is determination of metabolites, blood/plasma kinetics, organ/tissue collection, volatile compounds and use of infinite dose.

In vitro studies

As indicated in Section 3.3.5/3.3.6, the *in vitro* test guideline OECD 428 (2004c) presents general principles for measuring the dermal absorption and delivery of a test substance using excised skin. The principal of the test is that a test substance (preferably radiolabelled) is applied to the surface of a skin sample that separates two chambers of a diffusion cell. The upper half of the cell is usually referred to as the donor chamber whilst the lower half, containing an appropriate receptor fluid is known as the receptor chamber. The test sample is left on the skin for a predefined period of time which is either the length of the experiment or designed to reflect a likely time of exposure. The receptor fluid is sampled at regular intervals throughout the experiment. The removal of the test substance will be performed using a suitable washing procedure taking care not to damage the skin. The following paragraphs discuss points in the experimental design that should be taken into consideration.

Diffusion cell

Diffusion cells may be made of glass, PTFE or stainless steel (check that the test substance will not adsorb to the diffusion cell) and should provide a good seal around the skin, enable easy sampling and good mixing of the receptor solution in contact with the underside of the skin, and good temperature control of the cell and its contents. Both static and flow-through diffusion cells may be used. The exposed surface area of each sample should be as near identical as possible for any given experiment (typically ranges between 0.3 to 5 cm²). Consideration should be given to whether the donor chamber should be occluded or un-occluded depending upon the exposure scenario that is being modelled. Occlusion will generally lead to higher absorption values due to the increased hydration of the skin sample.

Receptor fluid

According to guideline OECD 428 (2004c) the use of a physiologically conducive receptor fluid is preferred although others may also be used provided that they are justified. Adequate solubility of the test chemical (not the test preparation) in the receptor fluid should be demonstrated so that it does not act as a barrier to absorption. According to the OECD guidance document (2004d) flow-through systems provide an adequate flow rate of receptor fluid that will prevent back diffusion although experience suggests that it is best to evaluate the solubility as indicated for static systems. In static systems it is particularly important to ensure satisfactory solubility. Ideally, an estimate of the likely maximum achieved concentration of test substance in receptor fluid should be made, based on previous *in vivo* or *in vitro* study data. Physicochemical data or experimental results should be used to show that about 10 times this concentration is achievable under the experimental conditions. The absorption rate profile over the study duration can be used retrospectively to confirm that solubility was not rate limiting. Suggestions regarding acceptable receptor fluids are made in the OECD (2004d) guidance document.

Skin

Skin from human or animal sources can be used as can reconstituted human skin models if data from reference chemicals are consistent with those in the published literature (OECD 2004d). Obviously the most relevant skin would be human but both rat (widely used test species) and pig (similar to human in terms of morphology and permeability) skins are also commonly used. Although viable skin is preferred, non-viable skin can also be used provided that the integrity of the skin can be demonstrated.

The human skin samples are generally from the abdomen or the breast (essentially due to availability) whilst rat skin samples are generally dorsal. The most common sites for pig skin are the flank, back, limb or ear.

Either epidermal membranes (enzymatically, heat or chemically separated) or split thickness skin (typically 200-500 μm thick) prepared with a dermatome, are acceptable. Should metabolic activity be suspected of playing a role in the absorption of a compound then fresh, metabolically active/ viable skin could be used. However, care will need to be taken to demonstrate that the skin samples can be expected to remain sufficiently metabolically active for the duration of the test. Fresh split thickness skin is metabolically competent but epidermal membranes have limited metabolic capacity. This may be of substantial influence in cases where metabolism plays a crucial role in dermal uptake (Boogaard et al, 2000). Full thickness skin may be used but excessive thickness (ca >1 mm) should be avoided unless specifically required for determination of the test chemical in layers of the skin. The selection of species, anatomical site and preparative technique must be justified. Acceptable data from a minimum of four replicates per test preparation are required.

Skin preparation integrity

It is essential that the skin is properly prepared and the integrity of the prepared skin must be checked. According to the OECD guidance document (2004d), the first step in evaluating a skin preparation is to make a visual examination for physical damage, excluding unsuitable samples. Secondly, the skin sample must be mounted and allowed to equilibrate in the cell and allowed to hydrate before testing. It is recommended that any pre-study integrity evaluation procedure does not take longer than a few hours, and is performed just before application of the test preparation. Acceptable methods of integrity evaluation include checking that electrical resistance to an alternating current, at up to 2 volts, is in the normal range for the skin type, checking that trans-epidermal water loss (TEWL) from the *stratum corneum* is in the normal range for the skin type or measuring the penetration characteristics of a reference material (e.g. tritiated water). However, any physical deterioration in the skin preparations (due to time at ambient temperature or hydration) may result in an overestimate of permeability. Pre-study evaluation has the advantage that damaged skin can be eliminated before performing the test. It is essential to remove any liquid used and to allow the skin surface to dry before application of test preparation. The skin should be allowed to equilibrate with the receptor fluid for typically 10 to 30 minutes before dose application.

When skin metabolism is being investigated, freshly excised skin should be used as soon as possible, and under conditions known to support metabolic activity. As a general guidance, freshly excised skin should be used within 24 hours, but the acceptable storage period may vary depending on the enzyme system being investigated and storage temperatures.

Test substance/compound

Ideally, the test substance should be radiolabelled. The radiolabel should be in a metabolically stable position preferably with ^{14}C , and of suitable radiochemical purity (ideally > 98%; OECD, 2004d). Other radioactive isotopes such as ^{35}S , ^{36}Cl , and ^{113}Sn or stable isotopes such as ^{15}N and ^{18}O may be used, particularly if the element is responsible for, or is a part of, the toxic portion of the compound. The radiolabelled chemical may be diluted, when appropriate, with non-radiolabelled chemical. The specific activity and radiochemical purity of the test substance must be known.

Using radiolabelled test substance is not always possible in which case a non-radiolabelled chemical may be used if a suitable, sufficiently sensitive and validated assay procedure exists for the chemical in the relevant samples.

Test preparation/vehicle/solvent

The test substance preparation (e.g. neat, diluted or formulated material containing the test substance which is applied to the skin) should be the same (or a realistic surrogate) as that to which humans or other potential target species may be exposed. Dilutions are made with the field vehicle, usually water, to produce a solution or suspension. In cases where exposure is to the chemical and not a formulation or dilution thereof, a neutral suspending agent which does not interact with the test chemical or affect the permeability of the skin, such as carboxymethylcellulose, may be used. However, organic solvents or special solubilising/suspending agents must not be used. Any variation from the 'in-use' preparation must be justified.

Normally, more than one concentration of the test substance is used in these studies, spanning the realistic range of potential human exposures.

Care must also be taken to ensure that sufficient quantities of radiolabel are present in each dose to allow sufficient sensitivity for that dose. The OECD guidelines indicate that a sensitivity of 1% dose is reasonable while the US EPA guideline indicates that the sensitivity required for a dose depends upon how small a quantity of the test compound is considered necessary to produce a toxic effect. It is also necessary to measure the actual concentrations obtained and to demonstrate that the dosing material is homogeneous.

Application to the skin

Under normal conditions of human exposure to chemicals, finite doses are usually encountered. Therefore, an application that mimics human exposure, normally 1-10 mg/cm² of skin for a solid and up to 10 µl/cm² for liquids, should be used. Some exposure scenarios may require applications to the skin surface to be infinite, i.e. where large volumes per unit area are applied.

These infinite dose experiments, with typical doses of $100 \mu\text{l}/\text{cm}^2$ (or $> 10 \text{ mg}/\text{cm}^2$) and more, may be appropriate to obtain steady-state conditions from which the steady-state flux or absorption rate and the K_p value (cm/hour) can be calculated. Such a volume ensures continuous excess of test preparation in the donor compartment.

According to the OECD guidance document (2004d) and the US EPA test guideline (1998), solids, such as powders, should be moistened with water to assist skin contact and quantitative and homogeneous application of the dose. Moistening will to some degree mimic the environmental humidity and the presence of perspiration as for normal 'in-use' conditions.

Experiments have demonstrated there is an inverse relation between concentration (area dose) and percentage of absorption. At low concentrations the absorbed test substance expressed as percent of applied dose per time interval is in general higher than the percentage absorption at high concentrations (although there are exception to this). As a consequence, there is no standard absorption percentage for a given substance. Therefore, dermal absorption studies should be done at different concentrations as a function of the expected use.

Occlusion Conditions

Generally an open atmosphere design is used for *in vitro* studies that allow a more direct comparison with the *in vivo* approach where the application site is normally protected but not occluded. Occlusion may be used if it reflects the human exposure scenario.

Temperature

The passive diffusion of chemicals (and therefore their skin absorption) is affected by temperature. The diffusion chamber and skin should be maintained at a constant temperature close to normal skin temperature of $32 \pm 1^\circ\text{C}$. Humidity should preferably be between 30 and 70%.

Duration of exposure and sampling

Skin exposure to the test preparation may be for the entire duration of the experiment or for shorter times (i.e. to mimic a specific type of human exposure). The most appropriate method of washing the skin is use of an aqueous soap, or that which reflects normal practice with the rinses being collected for analysis. The use of natural sponge or cotton swabs is recommended to more closely represent the mechanical surface contact associated with using soap.

A period of sampling of 24 hours is normally required to allow for adequate characterisation of the absorption profile. Since skin integrity may start to deteriorate beyond 24 hours, sampling

times should not normally exceed 24 hours unless data can be presented to justify a longer sampling time.

Terminal procedures

All components of the test system should be analysed and recovery is to be determined. This includes the donor chamber, the skin surface rinsing, the skin preparation and the receptor fluid/chamber. In some cases, the skin may be fractionated into the exposed area of skin and area of skin under the cell flange, and into epidermis and dermis fractions, for separate analysis.

Skin fractionation may be performed to further define the localisation of the test substance within the skin, as required by the objectives of the study. Tape stripping can be used to remove *stratum corneum* from skin (e.g. 15-25 strips for human skin) although it can pose practical challenges for *in vitro* studies.

Analysis

In all studies adequate recovery should be achieved (the aim should be a mean of $100 \pm 10\%$ of the radioactivity and any deviation should be justified). The amount of test substance in the receptor fluid, skin preparation, skin surface washings and apparatus rinse should be analysed, using a suitable technique. Under certain circumstances, for example the test of a volatile substance that had to be trapped in a filter, recovery boundaries of $100 \pm 20\%$ may be acceptable. The reason for not meeting the goal of $100 \pm 10\%$ recovery should be explained in the report.

A full recovery may not be achievable with unlabelled test substances due to analytical constraints, which should be detailed in the study report.

The steady-state flux or permeability coefficient (K_p) is determined for infinite dose experiments and therefore total recovery is not relevant because the only important endpoint is the appearance of test substance in the receptor fluid.

An area of concern in the interpretation of *in vitro* study results is the presence of test substance in the various skin layers, i.e. absorbed into the skin but not passed into the receptor fluid. According to the DG Health and Consumers 222 rev 7 document (EC, 2004), it was noted that it is especially difficult to examine very lipophilic substances *in vitro*, because of their low solubility in most receptor fluids. By including the amount retained in the skin *in vitro*, a more acceptable estimation of skin absorption can be obtained.

Alternatively, if an *in vivo* study has been performed that demonstrates that the material in the *stratum corneum* is not available for absorption then it is reasonable to exclude this material from the absorbed fraction in the corresponding *in vitro* study.

In vivo studies

As stated in the OECD 427 test guideline (OECD, 2004e), the advantages of the *in vivo* method are that it uses a physiologically and metabolically intact system, uses a species common to many toxicity studies and can be modified for use with other species. The disadvantages are the use of live animals, the need for radiolabelled material to facilitate reliable results, difficulties in determining the early absorption phase and the differences in permeability of the preferred species (rat) and human skin. Animal skin is generally more permeable and therefore may overestimate human percutaneous absorption. For obvious ethical reasons the testing of caustic/corrosive substances is to be avoided.

Test substance remaining in the skin after wash-off will normally move over time by one of three processes, it may be sloughed off by desquamation; it may be ingested when the animal grooms itself; or it may diffuse into the animal to become systemically available. Suitable technical procedures will be required to prevent grooming of the treated site, and to prevent desquamated skin from falling into the urine/faeces collection system. This is crucial to avoid overestimation of the systemically absorbed dose.

Animal species

The rat is the most commonly used species, but alternative species having skin absorption rates more similar to those of human, can also be used according to the OECD guidelines whilst the US EPA test guideline requires the use of the rat (preferably the same strain as was used for toxicology and metabolism studies). Young adult healthy animals of a single sex with males as the default (only males for US EPA) sex unless the female is known to be more sensitive of commonly used laboratory strains should be employed. At the commencement of the study, the weight variation of animals used should not exceed $\pm 20\%$ of the mean weight.

Number of animals

A group of at least four animals of one sex should be used for each test preparation and each scheduled termination time. Each group of animals will be killed after different time intervals, for example at the end of the exposure period (typically 6 or 24 hours) and subsequent occasions (e.g. 48 and 72 hours).

Housing and feeding conditions

The temperature in the experimental animal room should be 22°C (\pm 3°C). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used and should be freely available together with an unlimited supply of drinking water. During the study, and preferably also during the acclimatisation the animals are individually housed in metabolism cages.

Preparation of animals

The animals are marked to permit individual identification and kept in their cages for at least five days prior to the start of the study to allow for acclimatisation to the laboratory conditions. Following the acclimatisation period, (and approximately 24 hours prior to dosing), each animal will have an area of skin in the region of the shoulders and the back clipped. The permeation properties of damaged skin are different from intact skin and care should be taken to avoid abrading the skin. Following the clipping (approximately 24 hours before the test substance is applied to the skin) the skin surface is gently wiped with acetone to remove sebum. An additional soap and water wash is not recommended because any soap residue might promote test substance absorption. The area must be large enough to allow reliable calculation of the absorbed amount of test chemical per cm² skin, preferably at least 10 cm².

Test substance and test preparation

See *in vitro* section of this Appendix.

Dose levels

Normally more than one concentration of the test substance is used in these studies, spanning the realistic range of potential human exposures. The applied concentrations are generally expressed in terms of 'g/l' for OECD guideline studies whereas the US EPA guideline required the applied concentration to be expressed in terms of 'mg/cm²'.

The US EPA guideline recommends at least three dose levels should be used. These dose levels should be at log intervals (i.e. 1.0, 0.1, 0.01 and 0.001 mg/cm²) and span the range of doses expected in field exposure. The maximum practical dose is on the order of 1 mg/cm² – larger doses tend to fall off the skin or exceed saturation of the absorption process. When only three doses are given, the highest dose should be on the order of 0.1 mg/cm².

Application to the skin

An application site of a specific surface area (approximately 5-10% of the animal surface, i.e. 10 cm² for a 200-250 g rat) is defined on the skin surface. An application system should be used which consists of a raised support within which the test formulation is applied, it must be designed such that it does not cause damage to the skin. The system must prevent the animal from interfering with the application site, while preventing any spreading of the chemical outside the defined area. A common procedure is for a ring (e.g. rubber O-ring, PTFE rings, disposable beakers, etc.) of an inert material to be securely glued to the skin over the clipped area before applying the test formulation using cyanoacrylate adhesive to confine the treated skin area. A known amount of the test preparation is then evenly applied to the site. If a spreader is used, it should be analysed for retention of material in order to determine the actual amount applied. This amount should normally mimic potential human exposure, typically 1-5 mg/cm² for a solid or up to 10 µl/cm² for liquids. Any other quantities should be justified by the expected use conditions, the study objectives or physical characteristics of the test preparation.

To mimic normal exposure conditions, the surface of the skin may be un-occluded, semi-occluded or occluded. Un-occluded conditions allow any volatile component of the test preparation to evaporate and the normal drying process to occur. In addition, un-occluded or semi-occluded conditions can avoid skin integrity damage caused by excessive hydration which may occur with occlusion. In practice, the semi-occluded approach is a good compromise (and complies with EPA the guideline) as it can also prevent any sloughed skin from being added to the cage wash or urine whilst reducing excessive hydration.

For infinite applications the application site should be occluded. In case evaporation of semi-volatile test substances reduces the recovery rate of the test substance to an unacceptable extent, it is necessary to trap the evaporated test substance in a charcoal filter covering the application device. It is important that any device does not damage the skin, nor absorb or react with the test preparation. The animals are returned to individual metabolism cages in order to collect excreta.

Duration of exposure and sampling

The duration of exposure is the time interval between application and removal of test preparation by skin washing. Following the OECD test guideline a relevant exposure period (typically 6 or 24 hours) should be used, based on the expected human exposure duration. According to the US EPA test guideline, a full study would use exposure durations of 0.5, 1, 2, 4, 10, and 24 hours. For an abbreviated study, designed for a single exposure scenario, the recommended minimal durations of exposure are 1, 10, and 24 hours.

Following the exposure period, the animals are maintained in the metabolism cages until the scheduled termination. The animals should be observed for signs of toxicity/abnormal reactions at regular intervals for the entire duration of the study. At the end of the exposure period the treated skin should be observed for visible signs of irritation.

The metabolism cages should permit separate collection of urine and faeces throughout the study. They should also allow collection of ^{14}C -carbon dioxide and volatile ^{14}C -compounds, which should be analysed when produced in quantity (> 5%). The urine, faeces and trap fluids (e.g. ^{14}C -carbon dioxide and volatile ^{14}C -compounds) should be individually collected from each group at each sampling time. If there is sufficient information that little or no volatile radioactive metabolite is formed, 'open' metabowls can be used.

Termination times should allow an estimate of absorption over the period of interest, to provide data on systemic exposure per hour or per day. A 24-hour termination time point will allow an estimation of the daily systemic exposure. Other groups maintained for longer periods post-exposure are commonly necessary to understand the fate of the skin residue post-exposure. A difference between the OECD and US EPA test guidelines is that the OECD test method assumes a fixed exposure period (6-8 hours) and further termination times to investigate the depuration of radioactivity following the washing of the dose site. The US EPA test guideline for the main experiment stipulates that the test groups are terminated at the end of the exposure periods (e.g. 0.5, 1, 2, 4, 10, and 24 hours).

Following the OECD procedure, excreta are collected during the exposure period, up to 24 hours after the initial skin contact and then daily until the end of the experiment. Whilst three excreta collection intervals will normally be sufficient, the envisaged purpose of the test preparation or existing kinetic data may suggest more appropriate or additional time points for study.

At the end of the exposure period, the protective device is removed from each animal and retained separately for analysis. The treated skin of all animals should be washed at least 3 times with a cleansing agent using suitable swabs. Care must be taken to avoid contaminating other parts of the body. The cleansing agent should be representative of normal hygiene practice, for example an aqueous soap solution. Finally, the skin should be dried. All swabs and washings must be retained for analysis. A fresh cover should be applied to protect the treated site of those animals forming the later time point groups prior to their return to individual metabolism cages.

Terminal procedures

For each group, the individual animals should be killed at the scheduled time and blood collected for analysis. The protective device or cover should be removed for analysis. The skin from the application site and a similar area of non-dosed, clipped skin should be removed

from each animal for separate analysis. The application site may be fractionated (tape stripping procedure) to separate the *stratum corneum* from the underlying epidermis to provide more information on the test chemical disposition. The determination of this deposition over a time course after the exposure period should provide some indication of the fate of any test chemical in the *stratum corneum*.

Any potential target tissues may be removed for separate measurement before the residual carcass is analysed for absorbed dose. The carcasses of the individual animals should be retained for analysis. Usually analysis of the total carcass content will be sufficient. Urine present in the bladder at scheduled kill should be added to the previous urine collection. After collection of the excreta from the metabolism cages at the time of scheduled kill, the cages and their traps should be washed with an appropriate solvent. Other potentially contaminated equipment should likewise be analysed.

Analysis

In all studies, adequate recovery (i.e. mean of $100 \pm 10\%$ of the radioactivity) should be achieved. Recoveries outside this range must be justified. The amount of the administered dose in each sample should be analysed by suitably validated procedures. Statistical considerations should include a measure of variance for the replicates for each application.

The calculation of the percentage dermal absorption from *in vivo* studies is dependent on the sampling time. If sampling is done over a sufficiently long period of time (e.g. until serial non-detects in excreta), the amount detected in the application site after washing should not be included in the amount absorbed. In case excretion of the substance and/or its metabolites has not come to an end within the sampling period, but there are indications of a clear decrease in excretion, only a part of the skin bound dose may be included in the absorption by, for example, excluding the material in the *stratum corneum* if it was sampled separately. In case the experiment is terminated before serial non-detects in excreta are observed and/or no clear decline in excreta is measured, the amount located in the skin should be considered as being absorbed.

Finite vs. infinite doses

An 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, 2004d). In principle, the application volume should be large enough that the concentration of the chemical is not depleted. The main skin absorption parameters determined in an infinite dose study are the steady-state flux and the permeability coefficient.

Under the conditions of a finite dose, the maximum absorption rate may be reached for a period of the time, but it is not maintained, or it may not be achieved (OECD, 2004d). The concentration of the chemical in the donor fluid changes due to uptake of the chemical into the skin or evaporation, and it may also change (increase) due to evaporation of the donor fluid. The finite dose study enables the estimation of both maximal absorption rate (maximal exposure) and total absorption (overall exposure).

b) EPA-OSHA test rule

In 2004, after more than a decade of discussion and debate, the US EPA promulgated a final test rule under the Toxic Substances Control Act (TSCA) requiring *in vitro* dermal penetration rate testing for a number of chemicals. For the 34 chemicals listed in the test rule, it was intended that the results would be used by the Occupational Safety and Health Administration (OSHA) to evaluate the need for skin designations. Skin designations are intended to be a warning that dermal absorption, in addition to inhalation, may contribute to the expression of systemic toxicity. The *in vitro* dermal permeability coefficient (Kp) and short-term absorption rates (10 and 60 minutes) generated under the test rule are intended to provide quantitative data that could be used to estimate systemic burden following dermal exposure to chemicals.

An evaluation of the results from the US EPA's mandated *in vitro* dermal rate testing has been conducted (Fasano and McDougal, 2008). Permeability coefficients for chemicals applied in a water vehicle were well correlated with Potts and Guy (1992) correlation model; model Kp values for chemicals applied in isopropyl myristate (IPM) vehicle or when applied neat were not predictive of actual results. The 10- and 60-minute absorption data, more typical of workplace exposures to finite doses, were generally higher at the 10-minute exposure interval than at 60 minutes, due to inclusion of skin (radioactive) equivalents in the flux calculations (receptor fluid + skin equivalents ÷ exposure area ÷ exposure time). The Kp determinations from aqueous solutions and the flux measurements for neat chemicals will be useful for making estimates of skin absorption from realistic exposure scenarios. Kp measurements for chemicals applied in IPM vehicle are not envisioned to provide useful information for estimating the dermal exposure risk from chemicals.

c) Alternative dermal absorption models

Huski model

Given the current regulatory climate where new human *in vivo* studies are unlikely to achieve acceptance, an alternative model has been proposed that potentially allows the investigation of

dermal absorption through human skin samples in an *in vivo* situation. This model involves the use of athymic nude mice onto which ca. 1 cm² circles of full thickness human skin have been grafted (Capt et al, 2007). This *in vivo* study can then be run following the guidelines given for the rat *in vivo* studies and may be particularly interesting for obtaining a clearer estimate of the fate of more lipophilic compounds than may be possible with *in vitro* techniques.

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