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Guidance document on the estimation of dermal absorption according to a tiered approach: an update

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Summary

This report provides guidance to the estimation of dermal absorption in man at specific working conditions. It is meant for those involved in the health risk assessment of chemicals, *i.e.* industries and governmental agencies. The report is based on current experience using TNO-report V94.129 'Protocol for the estimation of dermal absorption according to a tiered approach on behalf of the risk assessment of pesticides' (Stevenson et al., 1994) as a starting point.

A tiered approach for the assessment of dermal absorption is described. The order of the tiers is determined by the simplicity of the studies on the one hand, and the relevance of the results for the estimation of human dermal absorption on the other hand. In general, the lower the tier, the more limited the data available are. As a consequence, the higher the tier, the more accurate the estimation of the dermal absorption. By introducing 'reasonable worst case' estimations, the results from the lower tiers will not underestimate the dermal absorption.

Tier 1
In tier 1, a default value of 100% for dermal absorption is used to calculate a dermal health-based acceptable occupational exposure level (the OEL_{dermal}), when no data are available.

Tier 2
In case health risks are indicated in tier 1, an estimate of the percutaneous penetration should be made. Data to be considered to estimate dermal absorption are data on the physico-chemical properties of the substance and/or data on oral absorption. The use of mathematical skin permeation models based on physico-chemical properties is discussed.

Tier 3
Experimental data derived from both *in vivo* studies with experimental animals and/or human volunteers, and *in vitro* studies with human and animal skin are used in tier 3 to further refine and quantify dermal absorption in man. It is noted that the results of *in vivo* as well as *in vitro* dermal absorption studies are heavily influenced by experimental conditions.

Contents

1. Introduction

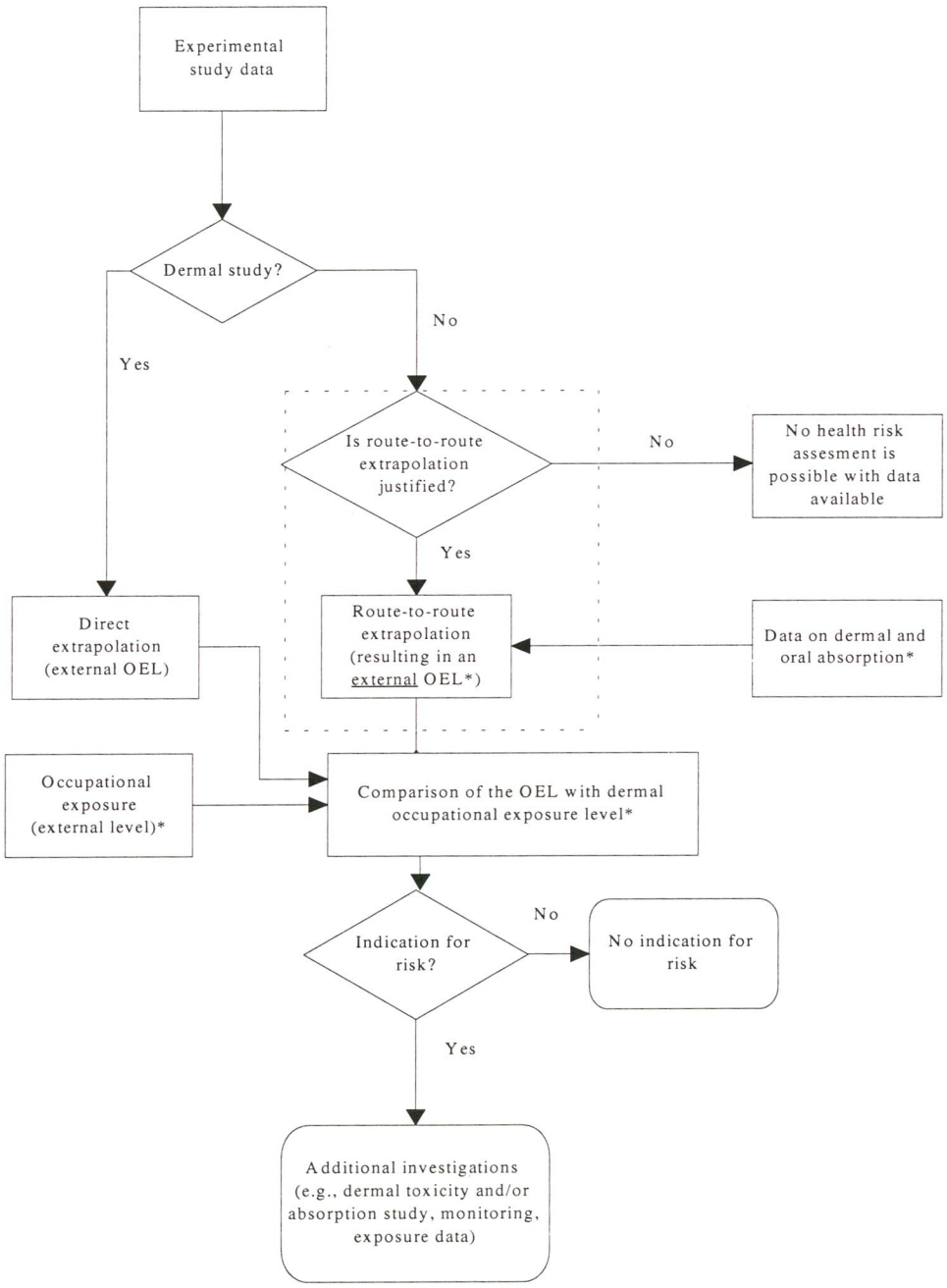
The occupational health risk of a chemical is assessed by comparing occupational exposure levels and the hazardous properties of the chemical. Exposure levels are estimated with models or based on actual measurements. Results from toxicity studies are translated in health-based acceptable occupational exposure levels (OEL), *i.e.* the Health-Based Occupational Reference Value (HBORV) for new and existing chemicals, and the Acceptable Operator Exposure Level (AOEL) for pesticides. In general, the toxicological studies available are performed by oral administration of the substance. This is satisfactory for the establishment of, *e.g.*, the Acceptable Daily Intake (ADI), but in the occupational setting people are exposed especially through skin contact and by inhalation. Therefore, occupational risk assessment has to be focussed on these exposure routes.

In Figure 1.1, the steps involved in the health risk assessment following dermal exposure are presented. In the dashed frame, route-to-route extrapolation is indicated. When oral studies are used for the assessment of a dermal OEL, route-to-route extrapolation will be necessary to bridge the gap between available data and the occupational situation. So far, route-to-route extrapolation only accounts for the differences in absorption via the routes of interest (Wilschut et al., 1998).

This report
This report provides guidance to the estimation of dermal absorption in man in relation to the outcome of the occupational health risk assessment, and is meant for those involved in the health risk assessment of chemicals, *i.e.*, industries and governmental agencies. By using a tiered-approach, guidance is given to a refinement of the estimate of dermal absorption when health risks are indicated. It is based on current experience using TNO-report V94.129 'Protocol for the estimation of dermal absorption according to a tiered approach on behalf of the risk assessment of pesticides' (Stevenson et al., 1994) as a starting point. After publication, that report was sent for comments to colleagues from academia, industries and governmental and governmental-liaised bodies in Europe and North-America, in order to reach consensus on the establishment of dermal absorption values in risk assessment of pesticides. The comments received and suggestions for modifications made (Stevenson et al., 1996), are used in this report.

In Chapter 2, the aim of this report is further substantiated. A general description of the tiered approach and the individual tiers is presented in Chapter 3. It should be noted that background information on the tiers is provided in the appendices of this report. They further elaborate the choices made. Conclusions and recommendations for further research are presented in Chapter 4.

Figure 1.1 Schematic presentation of health risk assessment following dermal exposure



* In case route-to-route extrapolation is supposed to end up in an internal value, the external occupational exposure level has to be extrapolated to an internal level by means of data on dermal absorption.

2. Aim of the guidance on dermal absorption

For an assessment of the occupational health risk of a chemical, the hazard has to be compared with data regarding the possible contact with the substance (exposure). In order to estimate the hazard of the substance, occupational exposure levels (OEL) are calculated from No-Observed-Adverse-Effect-Levels (NOAEL) established in repeated-dose toxicity studies. Assessment factors are introduced, accounting for differences between experimental animal and man, intraspecies differences, differences between experimental and occupational exposure duration, the adverse character of the effect, the dose-response curve, and the confidence of the database (Hakkert et al., 1996). Because occupational exposure to chemicals concerns mainly contact with the skin, an external dermal OEL (OEL_{dermal}) or internal OEL ($OEL_{internal}$) should be established. The risk is evaluated by comparison of either the external OEL_{dermal} with the estimated or measured exposure levels, or the $OEL_{internal}$ with the internal exposure levels. In both cases, data on dermal absorption are indispensable.

In general, toxicity studies, which function as basis for the establishment of the OELs are performed by oral administration. Oral-to-dermal extrapolation is necessary to be able to use the results. Several factors determine the validity of such route-to-route extrapolation, *i.e.*, the possible differences in kinetics, in metabolic processes (*i.e.*, first pass effects), and in bioavailability. Usually, quantification of most of these differences is not possible and correction is only made for differences in bioavailability, which is relatively simple. The OEL_{dermal} is calculated from the oral NOAEL using the percentages of oral and dermal absorption. In TNO report V97.520 'Evaluation of route-to-route extrapolation in health risk assessment for dermal and respiratory exposure to chemicals' (Wilschut et al., 1998), it was tried to validate the current route-to-route methodologies. It appeared that the difference in absorption is not the only factor clarifying the differences in toxic potential after exposure to different routes. However, it was not possible to establish reliably a factor to account for other uncertainties in route-to-route extrapolation. In the Appendix of TNO report V97.520, an overview of general aspects on route-to-route extrapolation is given. Therefore, knowing the uncertainties introduced by route-to-route extrapolation, TNO follows the current procedure as applied in many frameworks, unless there are clear indications that route-to-route extrapolation is not justifiable, in other words route-to-route extrapolation is performed accounting only for differences in absorption between the routes of interest.

The absolute bioavailability after dermal exposure will be largely influenced by dermal area dose (amount applied substance per unit area of skin), size of exposed skin area, exposure time, vehicle, occlusion, etc. (see Appendix A). It is stressed, that a generally applicable dermal absorption percentage and thus a generally applicable OEL_{dermal}, comparable with MAC-/TLV- or ADI-values cannot be derived, because it is assumed that dermal absorption is influenced to a larger

extent by occupational exposure conditions than oral and inhalation absorption. The use of the OEL_{dermal} in another risk assessment, where exposure conditions deviate from the conditions for which the dermal absorption is determined, can result in over- or underestimation of risks. So, the OEL_{dermal} applied is closely connected with the anticipated exposure scenario.

It is noted that dermal absorption may be influenced by local toxicity caused by (repeated) skin contact with the substance of interest (see Appendix A). However, at present it is not possible to take the influence of local toxicity after repeated dermal exposure on percutaneous absorption into account. Furthermore, it is noted that dermal absorption studies performed in accordance with the current OECD draft guidelines (OECD 1996a, 1996b) only apply to single exposures.

Data on bioavailability after dermal absorption are not always available for risk assessment. A 'worst-case' assumption of complete absorption through the skin can be made. However, often this assumption leads to an unacceptable risk ratio. One method to refine the risk estimation is to provide better data on dermal absorption by investigations.

This report focuses on the assessment of percutaneous absorption in relation to the occupational health risk assessment. A tiered approach for the assessment of dermal absorption is described. The order of the tiers is determined by the simplicity of the studies on the one hand, and by the relevance of the results for the estimation of human dermal absorption on the other hand.

This approach is intended, both to give reasonable recommendations on requests for additional studies on dermal absorption when it is decided to refine the risk assessment in this way, and for the evaluation of dermal absorption studies submitted.

3. Description of tiers

3.1 Introduction

In this chapter, a general description of the establishment and/or refinement of the dermal absorption in health risk assessment is presented.

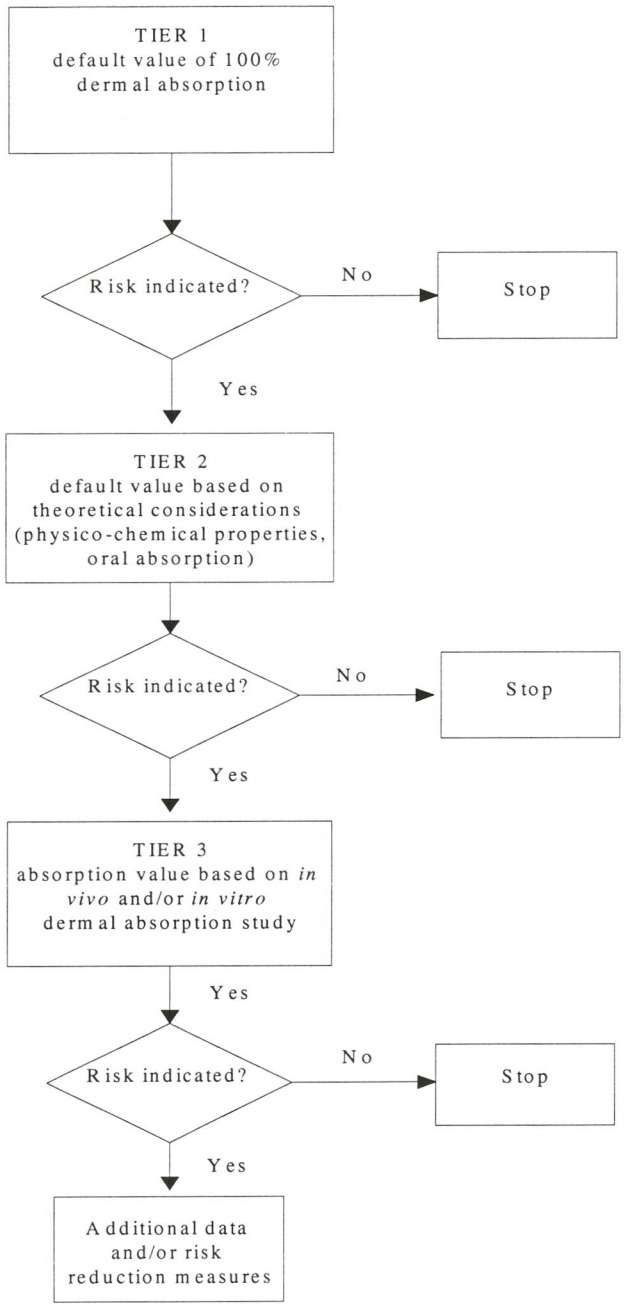
3.2 General description of tiers

A tiered approach for the estimation of dermal absorption is given (Figure 3.1). The sequence of the tiers is determined by 1.) the simplicity of the studies, and 2.) the relevance of the results.

In general, the lower the tier, the more limited the data available are. Alternatively, the higher the tier, the more accurate the estimation of the dermal absorption. By introducing 'reasonable worst case' estimations, the results from the lower tiers will not underestimate the dermal absorption.

It is not always necessary to have an accurate estimate of dermal absorption. Figure 1.1 presents how decisions for a request for the performance of (additional) dermal absorption studies should be made. The occupational health risk assessment may be based on 'worst-case' assumptions. When the risk estimation using these 'worst case' assumptions results in a risk ratio smaller than one or an acceptable 'margin of safety' (MOS), the occupational health risk is considered to be low. In this case, additional studies are not warranted. When the risk ratio exceeds one or the MOS is considered unacceptable, adverse health effects cannot be excluded. Additional investigations are necessary to verify assumptions. It has to be decided, which information is most critical regarding the validity of the risk assessment. The choice is determined by the assumptions made in hazard and exposure assessment, the simplicity of the additional investigations to verify the assumptions, and the results expected. The decision should be made on a case-by-case basis by expert judgement following the tiered approach and the subsequent need for additional data. The refinement of the estimate of dermal absorption is one of the choices that can be made. Alternatively, route-specific toxicity studies may be performed.

Figure 3.1 Guidance on the estimation of dermal absorption according to a tiered approach



3.3 Use of percentage dermal absorption versus the skin permeation coefficient Kp

As indicated before, the differences between oral and dermal absorption are taken into account when route-to-route extrapolation is performed. As a measure for dermal absorption, the percentage absorption is applied and used to convert internal to external dermal exposure levels. As long as this latter method is used in health risk assessment of chemicals, the percentage absorption is preferred to flux values or the skin permeation coefficient Kp. It is noted that flux or Kp values may be used, but that they first have to be translated to a percentage absorption value before they can be applied in the risk assessment process. Given the lack of insight in kinetics after dermal exposure has ceased, this should be done by means of a 'worst-case' approach assuming a 24-hour exposure and application of the calculated flux or the anticipated exposure level (expressed as dermal area dose) multiplied by the established Kp value (see also Appendix B).

3.4 Tier 1: default value

In tier 1, a default value of 100% dermal absorption is used to calculate the OEL_{dermal} or the internal exposure level. If this approach indicates the possible occurrence of unacceptable health risks, a substantiation of the dermal absorption may be one option to reduce the calculated risks (see Tier 2).

3.5 Tier 2: theoretical considerations

In case the use of a default value of 100% for dermal absorption (tier 1) results in an unacceptable risk, an estimate of the percutaneous penetration could be made by refining the applied value for dermal absorption by means of 1.) mathematical skin permeation models based on physico-chemical properties, and/or 2.) estimated default values for dermal absorption. Data to be considered to chose the default value for dermal absorption are data on the physico-chemical properties of the substance and/or oral absorption data. If after this substantiation an unacceptable risk is still indicated, a further refinement can be achieved by performing *in vivo* and/or *in vitro* dermal absorption studies (see Tier 3).

3.5.1 Mathematical skin permeation models

In the past years, many attempts have been made to relate physico-chemical properties to dermal absorption. Molecular weight (MW), octanol-water partition coefficient (log P), and water solubility (WS), are, amongst others, related with the capacity of a substance to permeate the skin. However, the use of models based on these parameters, either qualitatively or quantitatively in health risk assessment after dermal exposure, is questionable. In Appendix B this is further substantiated. It is concluded that given the variety in the experimental data on which the models

are based and the influence of this on the outcome of the experiment, and the lack of validation of these models, their general applicability is debatable.

3.5.2 Default values for dermal absorption

Physicochemical properties

Based on theoretical considerations on skin permeation, it might be expected that there should be an optimum in log P and a maximum in molecular weight for facilitating percutaneous absorption. Unfortunately, clear cut-off values for negligible, low and/or high dermal absorption of chemicals cannot be derived from the data presented in literature (see Appendix B). However, for the time being and based on the theoretics on dermal absorption and diffusion, the following criteria are chosen on a pragmatical basis to discriminate between chemicals with high and low dermal absorption:

- 10% dermal absorption is used in case MW > 500 g/mol and log P is smaller than -1 or higher than 4, otherwise
- 100% dermal absorption is used in case MW < 500 g/mol or log P between or equal to -1 and 4.

It is noted that the use of 100% dermal absorption is a 'worst-case' estimate. The lower limit value of 10% dermal absorption is chosen, because the data presented in literature indicate the occurrence of dermal absorption for tested compounds even beyond the extremes of log P and/or MW values. By expert judgement it is possible to deviate from 100 and 10% dermal absorption taken into account all data available, for example, data on ionogenic state, oral absorption and dermal area dose in occupational settings.

It is noted that the default values for dermal absorption are based on the same data on skin permeation as used for evaluation of the mathematical skin permeation models. However, the difference is that the default values only addresses to the upper and lower limits of skin permeation, where as the mathematical skin permeation models try to estimate the whole spectrum of dermal absorption by means of poorly validated methods.

Data on oral absorption

If a default value for dermal absorption of 100% is applicable based on the physico-chemical properties of a substance (see paragraph 3.5.2) and an appropriate oral absorption/ADME study is available, the results of the oral absorption study may be used to refine the default value for dermal absorption. It is required that the oral absorption is determined in bile-duct cannulated experimental animals, to get an accurate estimate of the oral absorption. Based on theoretical grounds and a comparison of oral and dermal absorption data available for 12 pesticides, it is assumed that dermal absorption will not exceed oral absorption established by means of bile duct cannulation (unpublished data). It is noted that the dose levels used in the oral absorption/ADME study should be relevant, *i.e.*, should approximate the NOAEL on which the risk assessment is based.

3.6 Tier 3: experimental data

Experimental data derived from both *in vivo* studies with experimental animals and human volunteers, and *in vitro* studies with human and animal skin, are used in tier 3 to quantify dermal absorption in man. It is noted that the results of *in vivo* as well as *in vitro* dermal absorption studies are heavily influenced by experimental conditions (reviewed in ECETOC, 1993). Two other examples of such conditions in view of risk assessment of dermal exposure are indicated in Appendix A. In the TNO reports V97.528 (Bosman-Hoefakker and Hakkert, 1997) and V98.356 (De Heer et al., 1999) the performance and evaluation of dermal absorption studies on pesticides have been discussed.

3.6.1 *In vivo* dermal absorption studies with experimental animals

In vivo dermal absorption studies performed in laboratory animals can be used to deduce percutaneous absorption in man at specific exposure conditions. It is important to note that the results of *in vivo* dermal absorption studies are heavily influenced by experimental circumstances, such as dermal area dose (in mg/cm^2), exposure duration, occlusion, vehicle, species, application site, etc. (see Appendix A). Therefore, test results can only be used with confidence if the experiment was tuned in to the exposure scenario of interest with regard to these aspects. However, in spite of these factors, a general scheme can be made of how to deal with the results of *in vivo* dermal absorption studies, even if performed under less relevant conditions with regard to dose and recovery. The diagram depicted in Figure 3.2 is applicable for the results obtained from *in vivo* studies to establish a dermal absorption value to be used in health risk assessment.
The first criterium for judging how to deal with the results of *in vivo* dermal absorption studies is the dose level (expressed as dermal area dose) applied in the experiment (Figure 3.2, Box 2). This aspect is of major importance since it is known that, at a constant exposure surface area, absorption, if expressed as a percentage of the applied dose (hereafter designated as %dose), decreases with increasing dose. If the dose levels applied in the *in vivo* study are at or below the dose levels anticipated to occur in the occupational situation, the left branch of the decision tree is applicable (Figure 3.2, Box 3). If higher than anticipated exposure levels have been tested in the *in vivo* experiment, the right branch of Figure 3.2 should be applied to prevent underestimation of dermal absorption (Figure 3.2, Box 6).

In both scenarios, the next aspect of importance is whether the sampling period following cessation of the exposure has been long enough to allow the animal to eliminate the (major part of the) absorbed test substance. This latter point is considered to be reached if at least 2 serial non-detects occur in excreta or if the excretion rate has clearly declined over several successive sampling times . If sampling has been performed until the excretion rate has (nearly) reached zero, the systemically available amount (either expressed as %dose (Figure 3.2, Box 4) or in absolute amounts (Figure 3.2, Box 7)) can be used to establish the dermal absorption. The amount systemically available is the cumulative amount detected

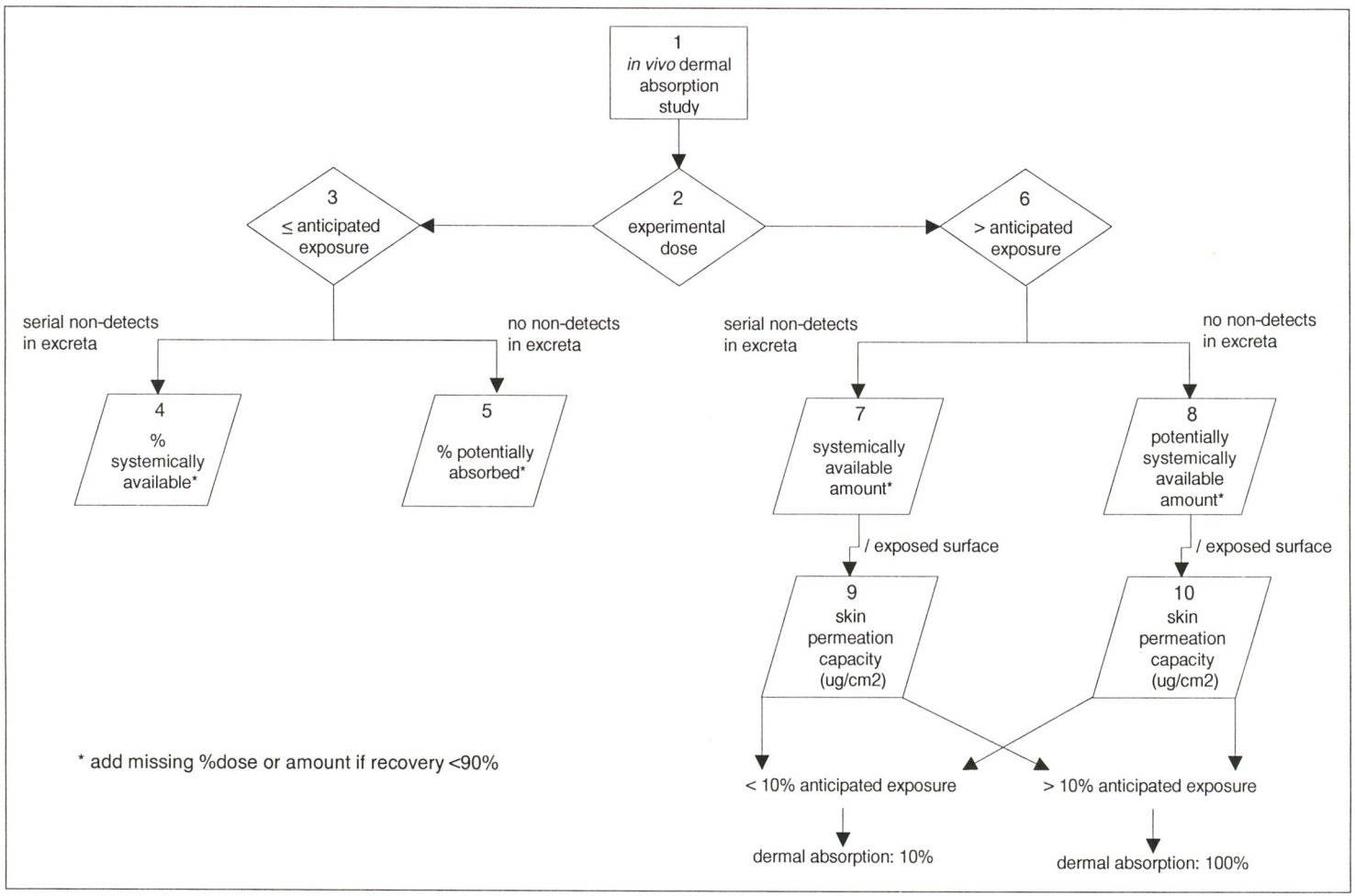
in excreta (including cage washes), blood, tissues, and carcass, but does not include the amount detected in the application site skin. In contrast, if excretion has not come to an end, it cannot be excluded that the amount retained in the application site skin may eventually become systemically available. Therefore, the potentially absorbed dose (the amount systemically available plus the amount in the application site skin) should be used as a value for dermal absorption in the latter case (Figure 3.2, Box 5 and 8), unless kinetics demonstrate that this is a clear overestimation.
Furthermore, if recovery is considered to be inadequate, this should be corrected for in case relevant dose levels have been tested. Ideally, recovery of the applied dose should be within 100±10% (OECD, 1996a). If this criterium for whatever reason cannot be met, but the study has otherwise been performed correctly, the missing %dose (or amount) should be added to the %absorption (or amount systemically available) determined in the *in vivo* study ('worst- case' approach).

In conclusion, 4 different scenarios of how to deal with *in vivo* dermal absorption data can be deduced from the diagram depicted in Figure 3.2:

1. The study includes dose levels relevant for the anticipated exposure scenario and the sampling period following the cessation of actual dermal exposure has been long enough to conclude that the skin residue dose does not become systemically available (*e.g.*, 2 serial non-detects in excreta). In this case, the % systemically available can be used as an estimate for dermal absorption, either or not corrected for incomplete recovery of the test substance.
   In Figure 3.2, the following route is applicable: 1-2-3-4.
2. The study includes relevant dose levels, but sampling was stopped before non-detection took place. In this case, the %dose detected in the application site skin should be regarded as being absorbed and the potentially absorbed dose, either or not corrected for incomplete recovery, should be used as a value for dermal absorption of the test substance. However, in case kinetics shows that the excretion rate diminishes, a value lower than the potentially absorbed dose may be established as a measure for dermal absorption by expert judgement.
   In Figure 3.2, the following route is applicable: 1-2-3-5.
3. The study does not include dose levels relevant for the exposure scenario under study (*i.e.*, higher dose levels), but sampling was continued until serial non-detection in excreta. In this case, the absolute amount that became systemically available, either or not corrected for incomplete recovery, should be (calculated and) used. This absolute amount should be divided by the exposed surface area and the resulting 'skin permeation capacity' should be compared with the anticipated exposure (dermal area dose) to conclude whether the skin permeation capacity may exceed the anticipated exposure. If the skin permeation capacity exceeds the level of 10% of anticipated exposure, an arbitrary dermal absorption value of 100% should be applied, and if not, an arbitrary dermal absorption value of 10% should be used
   In Figure 3.2, the following route is applicable: 1-2-6-7-9.
4. The study does not include relevant dose levels and the sampling period after cessation of exposure was too short to get insight in the fate of the amount of

the substance present in the skin. In this case, it cannot be excluded that the amount still present in the application site may eventually become systemically available, and is thus regarded as being absorbed. The absolute potentially systemically available amount, either or not corrected for incomplete recovery, divided by the exposed surface area is used to establish the 'skin permeation capacity' for the compound. This skin permeation capacity is subsequently compared to the anticipated exposure levels (dermal area dose) and a default dermal absorption value of 10 or 100% is chosen depending on the ratio between skin permeation capacity and anticipated exposure.
The following route is followed in Figure 3.2: 1-2-6-8-10.

Figure 3.2 Tier 3: Choice of percentage dermal absorption based on *in vivo* dermal absorption studies with experimental animals



3.6.2 *In vitro* dermal absorption studies with human and/or animal skin

In vitro studies, if conducted according to experimental conditions fitting occupational exposure conditions and correctly interpreted, can give data which may adequately reflect those from *in vivo* experiments. Preferably *in vitro* studies should include reference compounds (*e.g.*, caffeine, inulin, testosterone, hydrocortisone) to increase the applicability of the results. For now, the use of *in vitro* dermal absorption data for risk assessment purposes of chemicals in a quantitative way, however, awaits the results of proper validation of *in vitro* dermal absorption test methods (Howes et al., 1996). At present, *in vitro* results in itself can therefore not be used as an alternative to *in vivo* dermal absorption. If appropriate dermal penetration data are available for rats *in vivo* and for rat and human skin *in vitro*, the *in vivo* dermal absorption in rats may be adjusted in light of the relative absorption through rat and human skin *in vitro*. The latter adjustment may be done because the permeability of human skin is often lower than that of animal skin (*e.g.*, Barber et al., 1992; Bartek et al., 1972; Garnett et al., 1994; Howes et al., 1996; McDougal et al., 1990; Sato et al., 1991; Scott et al., 1987). A generally applicable correction factor for extrapolation to man can however not be derived, because the extent of overestimation appears to be agent and animal specific (Bronaugh and Maibach, 1987; ECETOC, 1993).

Thus, until further notice *in vitro* studies can only be used for semi-quantitative comparison of absorption of chemicals between species, between compounds within one species, and between different vehicles within one species. In this regard, it is important to realize that *in vitro* studies give relative results, *i.e.* that they should in first instance be compared with results generated within the same test system. This holds true both for the diffusion cells used as well as the skin preparation applied (*i.e.*, full-thickness skin, dermatomed skin, or epidermal membranes). An important aspect of *in vitro* studies is whether the dose levels applied are infinite or finite. Dose levels are infinite in case the applied dose presents itself in a constant concentration and is not rate limiting for the flux. After a lag time, steady state conditions with regard to flux are reached. If the applied dose is in a finite concentration and is rate limiting for the flux, no steady state is reached. Only in case a infinite dose has been applied, a proper Kp can be derived.

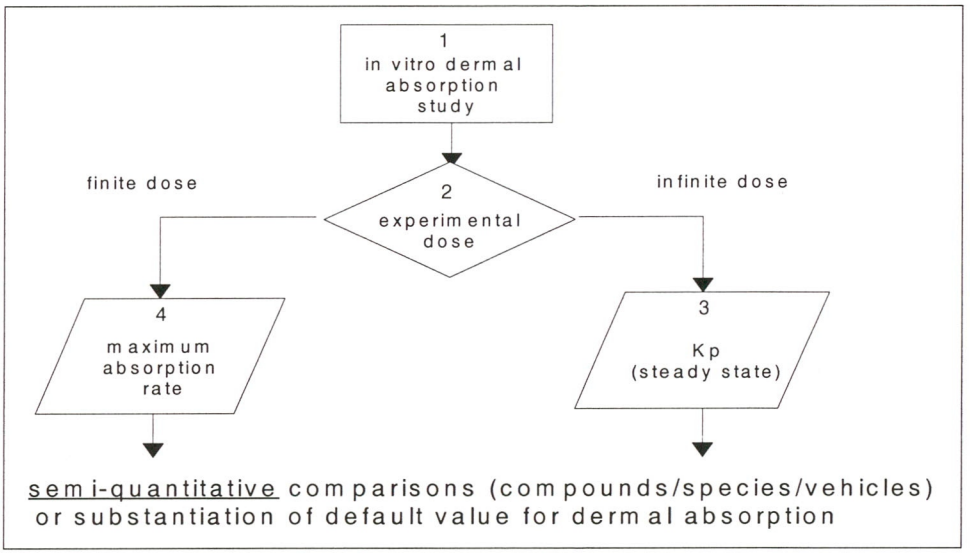
The maximum flux (derived from the linear part of the absorption vs time curve) should be used to semi-quantitatively compare absorption based on finite dose experiments (Figure 3.3). In this case, attention should be paid to the differences in flux at relevant exposure levels. For example, if at 200 $\mu g/cm^2$ the flux through rat skin is 10 times higher compared to human skin, but fluxes are comparable at the more relevant dose level of 20 $\mu g/cm^2$, there should be no correction for differences in skin permeability in health risk assessment.
Semi-quantitative comparison of data between species, compounds, or vehicles, should be made on the basis of flux values at relevant concentration or Kp values (in cm/hr; calculated by dividing the steady state flux by the concentration of the applied chemical) in case of infinite dose experiments (Figure 3.3), since this value is established under steady state conditions. The use of flux values at relevant

concentrations is preferred, because the infinite dose conditions on which the Kp is based do less resemble the dermal exposure in the risk characterisation.

Alternatively, *in vitro* data may be used to substantiate default values for dermal absorption if appropriate reference compounds have been included in the study.

In conclusion, *in vitro* dermal absorption data at present can only be used to assess semi-quantitative differences in dermal penetration between species, compounds, and/or vehicles provided the data have been generated within one test system and at comparable and relevant test conditions.

Figure 3.3 Tier 3: The use of dermal absorption data based on *in vitro* dermal absorption studies with human and/or animal skin



3.6.3 Human volunteer studies

There is no regulatory requirement for performing human volunteer studies addressing the dermal absorption of chemicals. However, the most reliable data for determining absorption through human skin are obtained from *in vivo* human volunteer studies performed under occupationally relevant test conditions (ECETOC, 1993). These data are preferable to animal data because no animal to human extrapolations are required. For technical and ethical reasons, however, the use of human volunteer studies is limited and the conduct of these studies is closely regulated (*e.g.*, World Medical Assembly Declaration of Helsinki, and ICH guidelines for Good Clinical Practice). A study protocol with supporting toxicological data must be submitted to an ethical committee and approval obtained before any study commences. The use of radio labelled compounds for human studies is subject to yet further regulation (ECETOC, 1983). Importantly,

with designing an experimental setup, it should be kept in mind that significant regional variations in percutaneous absorption occur that may be related to local differences in skin thickness and diffusivity (Grandjean, 1990; Wester and Maibach, 1989). To estimate dermal absorption, the results from the dermal experiment (primarily urine analysis) have to be corrected for the proportion of chemical excreted in urine after oral or (preferably) parenteral administration, although for ethical reasons the latter is rarely possible.

Other disadvantages of human volunteer studies are that they only measure absorption indirectly, metabolism data are required, analytical problems occur (low absorption, restricted use of radiochemicals), and the dermal and oral pharmacokinetics may not be the same due to differences in disposition and metabolism (ECETOC, 1993).

More information about the study protocol for *in vivo* human volunteer studies can be found in ECETOC (1993) and De Heer et al. (1999).

4. Conclusions and recommendations

4.1 Conclusions

- As a measure for dermal absorption, the percentage absorption is currently applied and used to convert internal to external dermal exposure levels. Flux or Kp values may be used but have to be converted to a percentage absorption value (by means of a worst-case approach) before they can be applied in the risk assessment process.

- The following tiered approach for the assessment of dermal absorption is used:
  tier 1 - a default value of 100% is applied to calculate a dermal health-based occupational exposure level when no data are available.
  tier 2 - in case health risks are indicated in tier 1, an estimate of dermal absorption is made on the basis of theoretical considerations. Data to be considered are data on the physico-chemical properties of the substance, and/or data on oral absorption.
  tier 3 - experimental data derived from both *in vivo* studies with experimental animals and/or human volunteers, and *in vitro* studies with human and animal skin are used in tier 3 to further refine and quantify dermal absorption in man.

- It is noted that the results of *in vivo* as well as *in vitro* dermal absorption studies are heavily influenced by experimental conditions. This is further elaborated in De Heer et al. (1999).

- The use of mathematical skin permeation models in the health risk assessment is not warranted because of the lack of validation of the underlying experimental data.

4.2 Recommendations

To further develop the tiered approach for dermal absorption in risk assessment of chemicals, validation of *in vitro* and *in vivo* dermal absorption studies is recommended. It is preferred that dermal absorption studies should include reference compounds (*e.g.*, caffeine, inulin, testosterone, hydrocortisone) to increase the applicability of the results, expecially in case of *in vitro* studies. Firstly, this highly contributes to a proper validation of the different types of studies, and secondly this increases the use of *in vitro* dermal absorption data at least in a semi-quantitative way (either for establishing of default values or for correction of *in vivo* dermal absorption data). In case validated dermal absorption studies are available, it is recommended to build a database on skin permeation for model building and validation, and/or justification of criteria for default values for

dermal absorption. By including data on physico-chemical properties in the database, the relation between skin permeation and physico-chemical properties can be studied and separate analyses for different chemical classes may eventually be performed.

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6. Signature

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Appendix A Factors determining dermal absorption

The results of *in vivo* as well as *in vitro* dermal absorption studies are heavily influenced by experimental conditions. The relevant experimental conditions are adequately reviewed by ECETOC (1993) and De Heer et al. (1999) and will not be dealt with in this appendix. In view of the risk assessment of dermal exposure two aspects of dermal exposure are addressed here:

- single vs repeated exposures: dermal absorption tests nearly always address the absorption of a test substance following a single exposure, whereas the occurrence of multiple dermal exposures is plausible within all occupational and possibly also other exposure scenarios. Experimental evidence suggests that there is no clear-cut relationship between dermal absorption and frequency of application. Repeated exposures may increase dermal absorption (*e.g.*, Roberts and Horlock, 1978; Wester et al., 1994) or may not affect the absorption characteristics (*e.g.*, Bucks et al., 1985a; Täuber et al., 1992; Wester et al., 1983). The experiments addressing repeated applications indicate that the results may be influenced by experimental conditions such as skin washing (Bucks et al., 1985b; Courtheoux et al., 1986) and direct effects of the test compound on the skin (Roberts and Horlock, 1978; Van der Valk and Maibach, 1989).

- irritation: skin irritation resulting from chemical exposure may influence the barrier function of the skin both *in vivo* and *in vitro*, either or not resulting in increased dermal absorption (Aungst, 1989; Ingram et al., 1993; Nangia et al., 1993; Wilhelm et al., 1991a; Wilhelm et al., 1991b; Xu and Chien, 1991). It was noted that the penetration enhancing effect of skin irritation was higher with hydrophilic compounds than with lipophilic compounds.
  In *in vivo* experiments in guinea pigs, the percutaneous absorption through damaged (tape-stripped) skin lacking stratum corneum greatly increased (3 to 10-fold) as compared with those of the intact skin. In the damaged skin, lag times were no longer observed and absorption occurred by simple diffusion in a pH-dependent way (Washitake et al., 1973). The results of this study suggest that the ability to build up a cutaneous reservoir was lost by removal of the stratum corneum, indicating that the stratum corneum is an important part in the cutaneous reservoir of chemicals (Washitake et al., 1973).

It is noted that generally it is assumed that substances showing no irritation in acute toxicity or irritation tests at high concentrations levels, are also not irritating at lower concentration levels after repeated exposure. However, it was shown in a study by Rennen et al. (1998) that the absence of an indication of local effects in any type of acute toxicity or irritation study does not exclude the occurrence of local effects upon repeated dermal or respiratory exposure.

Appendix B Mathematical skin permeation models and their use in dermal risk assessment

B.1 Introduction

In order to further develop tier 2, a literature search was started aimed at an evaluation of the estimation of dermal absorption based on physico-chemical properties. The following questions were relevant:
- Is sufficient scientific experience available to introduce mathematical skin permeation models based on physico-chemical properties in the health risk assessment for dermal exposure, and
- Is it possible to indicate physico-chemical properties that can be used for the selection of default values for dermal absorption, and
- If yes, is it possible to indicate clear ranges of these properties to justify the selection of default values for dermal absorption based on these characteristics?

B.2 Method

TOXLINE and MEDLINE were searched for literature published in the period 1990-mid 1998 with the following entries: dermal, skin, absorption, penetration, permeation, physico-chemical properties, molecular weight (MW), octanol-water partition coefficient (log P), model. This period was chosen because this report is based on Stevenson et al. (1994) and incorporates only recent developments.

The literature found was evaluated with regard to the following issues:
- definition of absorption;
- description of the applied model and outcome (percentage absorption, permeation coefficient (Kp));
- database on which the applied model is based; and
- validation.

These issues were considered relevant with regard to a proper evaluation of mathematical skin permeation models in health risk assessment for dermal exposure.

B.3 Results and discussion

B.3.1 General

Dermal absorption is determined by factors related to the substance, the skin, and the experimental conditions. Several skin layers have to be passed by a substance to become systemically available. For skin penetration, a substance has to diffuse into and through the (lipophilic) stratum corneum and the (hydrophilic)

(epi)dermis, into the dermal vasculature. The solubility in water is assumed to be rate limiting (Wilschut et al. 1995; ECETOC, 1997). Two diffusion routes are considered in the stratum corneum, *i.e.* the lipid (non-polar) and protein (polar) route. It is generally agreed that the preference for a route depends on the size and physico-chemical properties of the substance. Therefore, several authors have tried to relate physico-chemical properties with an estimation of dermal absorption retrieved from literature in so-called mathematical skin permeation models.

B.3.2 Mathematical skin permeation models

Reliable mathematical skin permeation models may be very helpful in risk assessment for dermal exposure, when relevant experimental data are not available. The application of these models is simple and straightforward. However, due to the complexity of the dermal absorption process, it may be doubted if these models are valid and generally applicable.

A large number of mathematical skin permeation models describing a relationship between dermal absorption and physico-chemical properties were found in literature (period 1990 up to mid 1998). An overview of the models based on physico-chemical properties, normally available in health risk assessment, is presented in table B.1. In this report, the use of these models is evaluated. The description of the models, the database on which they are based and their validation are discussed.

All models are more or less based on theoretical considerations on skin permeation, in combination with the 'best fit' of experimental skin permeation data in a mathematical relationship ('semi-empirical'). The models differ in the way they take into account all (*e.g.*, Wilschut et al., 1995), or some (*e.g.*, Guy and Potts,1993), of the routes described for skin permeation, the data they use, and the dependence of the skin permeation on physico-chemical properties.

Skin permeation coefficient

Nearly all mathematical skin permeation models presented in open literature in the period 1990-mid 1998 calculate the permeation coefficient (Kp, in cm/h). Theoretically, the Kp can be used in the estimation of dermal absorption in several ways, namely, as indication for high or low absorption, or to calculate the amount of substance absorbed (A_{abs}) according to the following equation:

A_{abs} (mg)	=	Kp (cm/h) x Exposure Time (h) x Exposed Skin Surface (cm^2) x Concentration of Substance in Vehicle (mg/cm^3)

A_{abs} (mg) can be used to estimate the percentage dermal absorption (%) in case the total amount applied on the skin is known. It is noted that the concentration of the substance in the vehicle may not exceed the maximum solubility of the substance in water, because it is assumed that with permeation through viable tissues of the skin, the water solubility is the rate-limiting factor. In other words, the solubility in

water is considered to be comparable with the solubility in the (epi)dermis (Wilschut et al., 1995; ECETOC, 1997).

Howes et al. (1996) presented a strategy for assessing percutaneous absorption based on Kp values. High penetration is considered likely in case the Kp exceeds 10^{-2} cm/h, and low penetration is expected in case the Kp is lower than 10^{-5} cm/h. However, details on these criteria are not presented.

It is noted that the models are based on steady state dermal absorption. During short exposure times, *i.e.*, in which steady state is not achieved, the mass of substance absorbed by the stratum corneum will be underestimated (Cleek and Bunge, 1993).

Underlying database
The models are generally based on *in vitro* dermal absorption data. Only Hagedorn-Leweke and Lippold (1995) based their model on experiments with human volunteers. At present, it is generally agreed upon that *in vitro* dermal absorption data can only be used semi-quantitatively to compare substances in their potential to permeate the skin provided they are tested under equal and relevant experimental conditions, because the outcome of an *in vitro* dermal absorption experiment is strongly influenced by the experimental design and does not reflect the *in vivo* dermal absorption. Hence, insight in the underlying database, and the definition and determination of dermal absorption is important in the evaluation of mathematical skin permeation models.

From the descriptions of the models (table B.1), it is not always clear on which data the model is based and how absorption is defined. Furthermore, the number of data on which the mathematical skin permeation models are based, is often limited and related to one specific experimental design, and/or for a certain group of substances. Tayar et al. (1991) established separate (and different) mathematical relationships for alcohols and steroid hormones, hydrocortisone 21-esters, and for phenolic compounds, based on *in vitro* data. Potts and Guy (1992), and Wilschut et al. (1995) used a more extensive dataset. The data were derived from different *in vitro* skin permeation experiments. However, they did not provide insight in the quality of the underlying database. It is noted that, for example, the data generated by Scheuplein et al. (1969) are generally used in fitting mathematical skin permeation models (MW 272-362 g/mol). Johnson et al. (1995) compared the skin permeation data available for six steroids and found that the measurements by Scheuplein significantly deviated from measurements by other groups. It was not clear what the reason is behind the factor 5 to 77 lower skin permeation coefficients determined by Scheuplein. Several possible sources of experimental error were evaluated. However, they did not appear to account for the observed discrepancies.

Validation

By studying the mathematical skin permeation models described in literature, it became clear that the models have often not been validated yet. In a study by Wilschut et al. (1995), five mathematical skin permeation models based on theoretical considerations about skin permeation and empirical skin permeation data, were validated. An extensive database was built (123 *in vitro* skin permeation coefficients in human skin, for 99 different substances in an aqueous solution; MW 18.0-764.9 g/mol; log P (-3.58)-5.58). It was concluded that three models provide reliable estimations of the skin permeation coefficient Kp, including the model Guy and Potts (1992) and Wilschut et al. (1995). The model of Fiserova-Bergerova et al. (1990) appeared to be less reliable (overestimation of Kp). It was recognised by the authors that differences in experimental settings influence the outcome. However, these differences were not taken into account in the model validation, because subselections on experimental design would result in too small groups for a reliable fit of the skin permeation models.

Opdam and Krüse (1996) compared the skin permeation coefficient Kp estimated with the model of Wilschut et al. (1995) with Kp experimentally determined in human volunteers for 7 substances (MW 86.2-209.2 g/mol; log P 1.6-3.9). Kp was overestimated by Wilschut et al. with a factor 2-4 for 4 compounds. For 3 substances, the estimated Kp was comparable with the experimentally determined Kp. However, the dermal exposure times in the human studies were too short to reach steady state. The human Kp values were estimated based on the mean residence time in the skin, the diffusion path length and the stratum corneum-water partition coefficient, and are not comparable with the Kp values as derived from the models.

B.3.3 Other data

Besides the mathematical skin permeation models described, additional literature on the relation between skin permeation and physico-chemical properties was found. In table B.1 only models based on log P and MW are presented. However, in literature other physico-chemical properties are also related with dermal absorption.

Potts and Guy (1995) showed a relationship between *in vitro* skin permeability and molecular volume, hydrogen bond donor and acceptor activity for 37 nonelectrolytes (molecular volume 10.6-114 cm^3/mol, log P (-1.4)-4.24). Hughes and Hall (1997) suggested that the ionization potential of the p-substituted functional group may have a pronounced effect on the dermal absorption of the compound. It was concluded that dermal absorption decreases when the substance is ionized. The lipophilicity of the similarly absorbed phenols did not influence the dermal absorption. Tayar et al. (1991) introduced the partition coefficient octanol-heptane as measure for H-bond donor acidity. However, the relation found by Tayar et al. was not supported in an *in vitro* skin permeation experiment reported by Bast (1997).

B.3.4 Selection of default values for dermal absorption based on physico-chemical properties

In Stevenson et al. (1994) default values for dermal absorption are selected based on log P and MW. At that moment, no scientific justification for these criteria was derived. The literature found, was studied for additional information on this subject.

Log P

In literature, optimum log P values are associated with relatively high dermal absorption.

Singh and Roberts (1994) studied the skin permeation of the nonsteroidal anti-inflammatory drugs in an *in vitro* dermal penetration study, using split-thickness human abdominal skin. A parabolic relationship was found between log P (range 0.05-4.42) and the log Kp. The optimal log P associated with maximum permeation was found to be around 3. Bast (1997) used the isolated perfused rabbit ear model to study dermal penetration through the intact skin. Substances (n=11; log P 0-5) dissolved in isopropyl myristate were applied to the skin during 4 to 6 hours, depending on the time required to reach steady-state conditions. It was found that relatively small molecules with log P between 0.1 and 2.3 penetrate the skin easily, while with increasing log P, skin penetration decreases. From log P 3.3, a distinct decrease was observed. However, substances with amphiphilic properties (n=4; log P 0.1-2.6), *i.e.* ionized at physiological pH, showed fast percutaneous penetration almost independent of the log P, when applied unionized in a lipophilic vehicle. Lee et al. (1994) studied the relationship between lipophilicity (log P (-0.95)-4.40) and skin permeation of 16 drugs in an *in vitro* skin permeation experiment. Skin from hairless female mice was used. It was shown that the relationship between skin permeation and log P depended on the vehicle used. A parabolic shape of the skin permeation versus log P was determined. It was found in this study that the peak (optimum log P) was at a more hydrophilic range (log P 0.19) compared with other references (log P 2-3). Lien and Gao (1995) determined an optimum log P of 2.5 for 17 non-steroidal anti-inflammatory drugs associated with maximum permeation (MW 138-358 g/mol; log P 0.77-4.88). It was not possible to determine an optimum log P by using 22 human skin permeation coefficients (MW 18-362 g/mol; log P (-1.38)-3.87). Morimoto et al. (1992) found a linear relation between Kp and log P for lipophilic drugs (log P: 0-3.94). However, Kp was almost constant for hydrophilic drugs and independent of log P (log P (-4.70)-0). It is not clear from the description by Lien and Goa (1995), how the skin permeation coefficients were determined.
Jackson et al. (1993) studied the hypothesis that dermal absorption should decrease with increasing Pow, considering the rate-limiting step as diffusion out of the stratum corneum through the viable epidermis. Male Fischer 344 rats were *in vivo* dermally exposed. The absorbed dose was defined as the total radioactivity found in excreta and tissues, including skin. Log P ranged from 2.13 to 7.97 (n=23). Except for two compounds, dermal absorption over 3 days after a single dose

decreased with increasing log P. The two outliners were explained by their different metabolism and elimination compared with the other compounds.

In contrast with the above studies describing a relationship between log P and skin permeation, Bronaugh et al. (1990) did not found a correlation between skin penetration and log P. They studied the *in vivo* dermal absorption of six fragrance ingredients in rhesus monkeys (MW 108-212 g/mol; log P 0.64-3.97). It was suggested that log P, i.e., solubility in octanol relative to solubility in water, fails to reflect the absolute water and lipid solubility determining the skin permeation, i.e., the actual lipid solubility of chemicals with high log P values may not differ substantially from that of chemicals with the lower log P values.

Molecular weight (MW)
The relation between skin permeation and MW is disputable. Within the ranges of molecular weight tested, no clear relation is established. A range from 137 up to 360 g/mol reported by Lien and Gao (1995; n=17) is considered too small to be related to skin permeation. Skin permeation was observed and associated with log P. This was also found by Sartorelli et al. (1998; n=11; MW: 128-323 g/mol) and Tayar et al. (1991).

Durkin et al. (1995) tried to find a method for estimating the absorbed dose from dermal contact with contaminated vegetation. For analysis, data (n=47; MW 60-452; log P (-2.82)-6.91) on dermal absorption in human volunteers determined by Feldmann and Maibach were used. It was found that no significant correlations between commonly available physico-chemical properties (Pow, molecular weight, water solubility, melting point and pK_a, or combination) and dermal absorption exist. Only for substances with a log P >1.85, the average dermal daily absorption over a five-day postexposure period could be estimated from the molecular weight (see table B.1).

Because of the difficulties in experimental testing, it is not possible to establish limits to dermal absorption for reasonably sized substances (Potts and Guy, 1992). Potts and Guy included substances up to MW 750 g/mol in their database. Skin permeation was observed up to this molecular weight in *in vitro* skin permeation experiments.

B.4 Conclusions

Mathematical skin permeation models
Based on the results of a literature search (1990 - mid 1998) on mathematical skin permeation models, it is concluded that insufficient scientific validation is available to introduce mathematical skin permeation models based on physico-substance properties in the health risk assessment for dermal exposure.

Despite the simplicity of the application of these models, it is not possible to use them quantitatively in health risk assessment. The main disadvantage is that these models are based on *in vitro* dermal absorption studies, while there is insufficient knowledge on the relationship between *in vitro* and *in vivo* dermal absorption. Furthermore, the present methodologies to measure skin permeation are not harmonized and it is known that differences in the experimental conditions will be reflected in the outcome of the test, *i.e.*, the skin permeation coefficient Kp. Insight in the quality of the database on which the models were based, was often unclear. A definition or description of the determination of dermal absorption, as well as the underlying database was generally poorly described. The number of skin permeation data on which a model was based, was often small and included only a specific group of substances. In case of the use of larger datasets, the influence of the different experimental settings on dermal absorption were not taken into account. The mathematical skin permeation models were often not validated. Only one validation study is described by Wilschut et al. (1995). However, the limitations of the data used to build the models (training set) are also applicable for the validation set used by them.

It is agreed with Howes et al. (1996), Sartorelli et al. (1998), and Leung and Paustenbach (1994), that mathematical skin permeation models based on physico-chemical properties can only be a tool in quantitative health risk assessment for dermal exposure, provided that the data they are based on data of sufficient quality. The use of the models, at this moment, is therefore restricted to only a semi-quantitative comparison of the skin permeation potential. It is agreed with Ridout et al. (1992) that for structurally related groups the models may be used to rank these substances on skin permeability potential taking into account the experimental conditions of the database on which the model is based. However, it is unclear how the results for one series of compounds can be applied to another.

Criteria for the selection of default values for dermal absorption

Based on theoretical considerations on skin permeation, it might be expected that there should be an optimum in log P and a maximum in molecular weight for facilitating percutaneous absorption. Unfortunately, clear cut-off values for negligible, low and/or high dermal absorption of chemicals cannot be derived from the data presented in literature (see Appendix B). However, for the time being and based on the theoretics on dermal absorption and diffusion, the following criteria are chosen on a pragmatical basis to discriminate between chemicals with high and low dermal absorption:

- 10% dermal absorption is used in case MW > 500 g/mol and log P is smaller than -1 or higher than 4, otherwise
- 100% dermal absorption is used in case MW < 500 g/mol or log P between or equal to -1 and 4.

It is noted that the use of 100% dermal absorption is a 'worst-case' estimate. The lower limit value of 10% dermal absorption is chosen, because the data presented in literature indicate the occurrence of dermal absorption for tested compounds even beyond the extremes of log P and/or MW values. By expert judgement it is

possible to deviate from 100 and 10% dermal absorption taken into account all data available, for example, data on ionogenic state, oral absorption and dermal area dose in occupational settings.

It is noted that the default values for dermal absorption are based on the same data on skin permeation as used for evaluation of the mathematical skin permeation models. However, the difference is that the default values only addresses to the upper and lower limits of skin permeation, where as the mathematical skin permeation models try to estimate the whole spectrum of dermal absorption by means of poorly validated methods.

Table B.1 Overview of mathematical skin permeation models reported in open literature (1990 - mid 1998)

Publication	Model description	Remarks
Tayar et al. (1991)	1. alcohols and steroid hormones: log Kp (cm/sec) = -7.15 + 0.16 log P r = 0.164 n = 22 log P: (-0.77) - 3.7 data: Scheuplein 1964, Scheuplein et al., 1969 2. hydrocortisone 21-esters: log Kp (cm/sec) = -8.88 + 0.80 log P r^2 = 0.88 n = 11 data on MW or log P: not provided data: Anderson et al. (1988) 3. phenolic compounds: log Kp (cm/sec) = -0.37 $(\log P)^2$ + 2.39 log P - 8.71 r^2 = 0.87 n = 18 data on MW or log P: not provided data: Roberts et al. (1977)	*Remarks of the reviewer* Absorption: no data, model was based on historical data Validation: no data *Remarks of the authors* Ad. 1 Log P cannot act as an unifying variable; MW did not correlate with permeability coefficients, and did not improve the correlation when taken together with log P. Ad. 2 log Kp did not correlate with log Koct-hept Ad. 3 a parabolic relationship Log Kp did not correlate very well with log Koct-hept. Correlation increased when compounds with log P >2 were removed. *Remark of the reviewer* Kp was determined in human epidermis or stratum corneum using a three-compartment model (aqueous donor compartment, epidermis or stratum corneum, aqueous acceptor compartment).

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Fiserova-Bergerova (1993)	Kp (cm/h) = (0.038 + 0.153 Pow) $e^{-0.016\,MW}$ data: unknown, based on Berner and Cooper (1987); from saturated aqueous solutions for non-polar compounds	*Remarks of the reviewer* Absorption: no data Validation: by Wilschut et al. (1995) and discussed by Bunge (1998) The model overestimates dermal absorption. *Remark of the author* Aim of model development was not to predict dermal absorption, but to develop critiria for the skin notation (Fiserova-Bergerova, 1993).
Durkin et al. (1995)	log AR (% of applied dose/day) = 1.5 - 0.04 MW AR = average daily absorption over a five-day postexposure period, for compounds with log P > 1.85 $r^2 = 0.67$ n = 47 (14 steroids, 21 organic substances, 12 pesticides) MW: 60.6 - 452.5; log P: (-2.82) - 6.91 data: Feldmann and Maibach (1969, 1970, 1974) (human volunteers; vehicle: acetone, 4 µg/cm², 2.8-20 cm², exposure duration: 24 hours, urine collection during 5 days)	*Remarks of the reviewer* Absorption: determined by urinary excretion, corrected for incomplete recovery. Validation: no data *Remarks of the authors* Regression analysis revealed no significant correlation between log P values, MW, or both, and absorption fraction or log absorption fraction for the total database. Additional variables as water solubility, melting point, pKa did not yield statistically significant correlations.

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Publication	Model description	Remarks
Hagedorn-Leweke and Lippold (1995)	log Kp (cm/h) = -2.46 + 0.38 log $P_{octanol\text{-}vehicle}$ (log $P_{octanol\text{-}vehicle}$ = -0.56 + 0.91 log P; r^2 = 0.99) r^2 = 0.90 n = 10 (miscellaneous compounds) log P: 3.07 - 6.51 data: human volunteers (*in vivo* experiments; vehicle: mixture of propylene and water; 14-16 cm^2 exposed, 2-6 ml with various concentrations)	*Remarks of the reviewer* Absorption: based on concentration decrease in vehicle Validation: no data *Remarks of the authors* The permeability was sufficient predicted by $P_{octanol\text{-}vehicle}$; *i.e.*, it seemed to be almost independent of the molar volume.
Hori and Ito (1995)	log Kp (cm/sec) = -6.26 + 0.67 log P - 0.0060 MW r^2 = 0.66 n = 76 (miscellaneous compounds) MW: 32 - 765; log P (-1.19) - (5.49) data: Taya et al. (1991); Itoh et al. (1990); Michaels et al. (1975); Hirvonen et al. (1991); Flynn (1985)	*Remarks of the reviewer* Absorption: not defined, model based on historical data Validation: no data Based on formula of Potts and Guy (1992)

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Publication	Model description	Remarks
Lien and Gao (1995)	non-steroidal anti-inflammatory drugs $\log R = -1.930 + 1.824 \log P - 0.370 (\log P)^2$ R = (% absorbed)/(% unabsorbed) $r^2 = 0.74$ n = 17 MW: 137.13 - 357.81; log P: 0.77 - 4.88 data: Yano et al. (1986); human skin permeation data (not further described)	*Remarks of the reviewer* Absorption: not defined, model based on historical data Validation: no data *Remarks of the authors* Due to the narrow MW range, addition of MW is not justified statistically. Ideal lipophilicity for maximum permeability: log P = 2.5
Wilschut et al. (1995)	Kp (cm/h) = 1/ ((1/(Kpsc + Kpol)) + (1/Kaq)) $\log Kpsc = -1.326 + 0.6097 \log P - 0.1786 MW^{0.5}$ $Kpol = 0.0001519/\sqrt{MW}$ $Kaq = 2.5/\sqrt{MW}$ n = 123 (miscellaneous compounds) MW: 18- 764.9; log P: (-3.58) - 5.58 data: human *in vitro* experiments (historical data, aqueous solutions)	*Remarks of the reviewer* Absorption: not defined, model based on historical data Validation: Model is validated with human *in vitro* data. The database was divided in a so-called training and validation set. These sets were used to estimate the regressions coefficient of the model. The residual variance was determined and compared. Opdam and Krüse (1996) compared the Kp estimated with Kp experimentally determined in human volunteers for 7 substances (MW: 86,2-209.2; log P: 1.6-3.9). Kp was overestimated by Wilschut et al. with a factor 2-4 for 4 compounds. For 3 substances, the estimated Kp was comparable with the experimentally determined Kp. The models is based on Robinson (1993).

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Publication	Model description	Remarks
Sartorelli et al. (1998)	Kp (cm/h) = 0.037 - 0.003 ln Pow r^2 = 0.82 n = 11 (8 PHCs and 3 organophsophorus compounds) MW: 128.2 - 322.5; log P: 0.7 - 5.9 data: *in vitro* experiments with monkey abdomen skin (static diffusion cells, without occlusion, full thickness skin, various concentrations, 1.77 cm^2, vehicle: acetone)	*Remarks of the reviewer* Absorption: amount of substance in samples obtained from the cells. Kp= absorption flux at steady state/concentration applied Validation: no data A more general model which also included MW and vapour pressure was evaluated as well, but made no substantial difference. Sartorelli et al. stressed the need for internationally accepted guidelines for *in vitro* experiments before using models based on physico-chemical properties for regulatory toxicology.

Table B.1 Overview of mathematical skin permeation models reported in open literature (1990 - mid 1998)

Publication	Model description	Remarks
Guy and Potts (1993); Potts and Guy (1992)	log Kp (cm/h) = -2.74 + 0.71 log P - 0.0061 MW r^2 = 0.67 n = 89 MW: 18 - 750; log P: (-3) - 6 data: Flynn (1990) (human *in vitro* epidermal permeability data)	*Remarks of the reviewer* Absorption: no data, model based on historical data Validation: described by Wilschut et al. (1995); It was concluded that the model provides reliable estimations of skin permeation for substances with a log P between -1 and 5. It was not possible to establish limits to dermal absorption for a reasonably sized nonelectrolyte, because the database did not contain any substances whose properties fit the criteria necessary to define the regime. This is not surprising since such compounds would be expected to be extremely water-insoluble and difficult to work with under the experimental conditions required to establish steady-state penetration kinetics across skin from an aqueous donor phase into an aqueous receptor phase (Potts and Guy, 1992).
Morimoto et al. (1992)	Kp (cm/sec) = $2.73\ 10^{-8}$ + $1.17\ 10^{-7}$ $Pow^{0.751}$ n = 16 (hydrophilic and lipophilic drugs) MW 130 - 357; log P: (-4.7) - 3.94 data: *in vitro* skin permeation experiments (donor and receiver: distilled water, stirring, split-thickness skin); duration of experiment: 10 h; human chest skin	*Remarks of the reviewer* Absorption: no data Validation: no data *Remark of the author* The relation between Kp en log P was linear for lipophilic drugs (log P $\geq$0), while for hydrophilic drugs the Kp was almost constant, ca. $2\ 10^{-8}$ cm/sec, and independent of log P.

TNO-VOEDING

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Titel : Rapport-stamblad

Rapportnr. : V 98.1237 Vertrouwelijk / Openbaar*)
Auteur(s) : Hees, Wilschut, Stevenson, Hakkert Afdeling: ATRA
Titel : Guidance document on the estimation of dermal absorption according to a tiered approach : an update
Projectnummer : 480361
Opdrachtgever : SZW
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TNO hoofdonderwerp en product code : _____

Publikatie code : dit is een rapport m.b.t. onderzoek volgens....
( ) 4.1 = eigen richtlijnen ( ) 4.2 = richtlijnen opdrachtgever
( ) 4.3 = standaardprotocollen (X) 4.4 = literatuurstudies, adviezen e.d.
Trefwoorden (max. 5): dermal absorption, risk assessment

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