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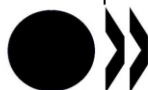

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GUIDANCE NOTES ON DERMAL ABSORPTION

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The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organisations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. UNDP is an observer. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

These *Guidance Notes on Dermal Absorption* (Guidance Notes) are intended to provide practical guidance to facilitate harmonised interpretation of experimental data from specific dermal absorption studies, where they are available, and to provide advice on alternative ways to estimate dermal absorption when there are no data or few specific data available. The Guidance Notes were prepared with the primary focus on establishing appropriate dermal absorption values for occupational health and public health risk assessment of pesticides and biocides. Some aspects of this guidance will also be relevant for other groups of chemicals, such as veterinary medicines and industrial chemicals, thus enabling a consistent approach. However, it is emphasised that most of references and examples of data in these Guidance Notes are related to pesticide chemicals.

The Guidance Notes consider the type of data that may be available to risk assessors for estimating or calculating the dermal absorption for the evaluation of public health or safety risks posed by a pesticide chemical. The Guidance Notes also provide guidance on the interpretation of such data to facilitate a harmonised approach.

It is recognised that regulatory authorities around the world currently have differences in the acceptability and use of certain types of data, and many regulatory authorities have their own guidance that should be consulted where applicable. In general, all available data or relevant information are considered in a weight-of-evidence approach to estimating a human dermal absorption value. It is beyond the scope of these Guidance Notes to provide a harmonised regulatory or scientific view on the use of specific types of data in those cases where regulatory authorities have differing data requirements.

These Guidance Notes outline core concepts and refer the reader to other useful sources when more detailed or specific information is required. These Guidance Notes are intended to complement OECD Test Guidelines and other publications by the OECD, especially TG 427 (*in vivo*) and TG 428 (*in vitro*) (OECD 2004a and 2004b) and the OECD *Guidance Document for the Conduct of Skin Absorption Studies* (OECD 2004c). These notes are also designed to complement the *WHO/IPCS Environmental Health Criteria 235: Dermal Absorption* (WHO 2006) and guidance documents developed by governments (*e.g.* EC 2004; EC 2006; and USEPA 1996 amongst others). All of these documents encourage a harmonised approach to the conduct of dermal absorption studies.

These Guidance Notes do not comprehensively address the issue of test methodology and study performance, recognising that there are numerous factors that can influence dermal penetration. TG 427 and TG 428 and OECD GD 28 (2004c) should be used when designing dermal absorption studies.

The OECD and WHO/IPCS documents listed above should be read in conjunction with these Guidance Notes. The WHO/IPCS (WHO 2006) document serves to introduce dermal absorption at a broader level, and the OECD Test Guidelines advise on the conduct of the studies. In contrast, these Guidance Notes are designed to help assess and interpret specific studies for the estimation of dermal absorption values.

While dermal absorption values form an integral part of the risk assessment process, these Guidance Notes do not address the entire risk assessment process. Although different regions and countries of the world may have different approaches to the type of data required for the assessment of public health and

occupational safety of compounds, these Guidance Notes do not attempt to reconcile these differences of approach.

The project started in 2005 with the establishment of an Australia led expert group on dermal absorption (EGDA) under the auspices of the Working Group on Pesticides (WGP). The original project was divided into two parts: (1) the drafting of guidance on analysis and evaluation of dermal absorption studies; and (2) the development of recommendations for the selection of default dermal absorption factors. Absorption of a chemical through human skin, following dermal exposure, determines the actual dose by the dermal route. In cases in which dermal absorption data for a chemical is unavailable, a default dermal absorption factor is used for estimation of dermal dose. The use of different dermal absorption factors by different regulatory authorities may affect the risk assessment outcome.

A first draft guidance on analysis and evaluation of dermal absorption studies was developed by the EGDA in August 2005.

At a face-to-face meeting held in Geneva in September 2005, the project lead recognised that there were significant differences between the approaches used in North America and in the European Union for dermal risk assessment. An interim working document dated November 2006 outlined these differences in order to facilitate further discussions on the development of a more harmonised approach. A second meeting was held in Paris in October 2007, and the EGDA agreed to refocus the scope of the project.

The draft *Guidance Notes for the Estimation of Dermal Absorption Values* were circulated for comments to the Working Group of National Co-ordinators of the Test Guidelines Programme (WNT) and WGP in May 2008. Comments were requested from the WNT on a second draft in October 2010. The revised document was approved by the 23rd Meeting of the WNT in April 2011.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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1. INTRODUCTION

1. Chemicals in workplaces or other environments may come into contact with the skin and be absorbed. Determining the extent of dermal absorption is a key step in the risk assessment of such chemicals. Many factors can affect the numerical value that is used to represent the degree of dermal absorption, such as exposure time, product formulation, dose, and the fate of the chemical in the skin. In addition, there are also differences in the way that national agencies interpret the available data. It should be noted that while dermal absorption studies are available for most pesticides, these studies are generally not available for most other classes of chemical.

2. The assessment of dermal absorption studies was identified as a technical issue that could be a constraint to international collaboration on the review of pesticide data. It was noted that guidance notes on interpreting dermal absorption studies and consideration of default values for dermal absorption would assist with technical harmonisation.

3. These Guidance Notes attempt to provide harmonised guidance to assist in the uniform interpretation of dermal absorption studies and guidance on estimating dermal absorption values in the absence of such studies. They were prepared with the primary focus on establishing appropriate dermal absorption values for occupational health and public health risk assessment of pesticides and biocides. This guidance may also be relevant for other groups of chemicals, such as veterinary medicines and industrial chemicals, thus enabling a consistent approach. The purpose of this document is to provide:

- An outline of the information that may be available for estimating dermal absorption.
- Practical guidance for using such information to estimate dermal absorption values.

4. Estimates of dermal absorption values are derived from experimental data *in vivo* or *in vitro*, or both. Such data allow for direct or indirect estimation of dermal absorption of a test substance through human skin. Part 1 of this document discusses issues that should be considered when evaluating such experimental data. Part 1 also includes a discussion on combining *in vivo* and *in vitro* data in the 'Triple Pack' approach. Part 2 of this document contains a general discussion on how to estimate the dermal absorption of a chemical in the absence of experimental data.

2. SUMMARY OF RECOMMENDATIONS

Part 1: Interpretation of dermal absorption studies

5. It is recognised that regulatory authorities around the world currently have differences in the acceptability and use of certain types of data, and many regulatory authorities have guidance that should be consulted where applicable. In general, all available data or relevant information are considered in a weight-of-evidence approach to estimating a human dermal absorption value. The confidence in any particular piece of information will determine the weight it is given in the overall risk assessment. The guidance presented in this document will assist in evaluating the level of confidence that can be given to any particular data.

6. *In vitro* studies (Section 4) should be conducted using OECD TG 428 (OECD 2004b) or a similar protocol. In addition to providing guidance on evaluation of such studies, this section also includes guidance on evaluating the acceptability of non-guideline studies:

- Currently, regulatory authorities around the world have differences in the acceptability and use of *in vitro* data. It is beyond the scope of these Guidance Notes to provide a harmonised regulatory or scientific view on the use of *in vitro* data for regulatory risk assessment.

- If it is to be used as 'stand alone' information, an isolated *in vitro* study on rat skin is unlikely to underestimate absorption, and it could provide a rough estimate that could replace a worst case default value (of usually 100%) in risk assessment.

- The weight of evidence suggests that the predicted dermal absorption in humans may be overestimated when the estimation is based on an *in vitro* study on human skin if all of the test substance retained in the skin (following washing) is included.

- The draft EFSA *Scientific opinion on the Science behind the revision of the Guidance Document on Dermal Absorption* reports on the outcome of an analysis of human *in vitro* data *vs.* dermal absorption values derived from a "triple pack" approach (EFSA, 2010 (draft)).

7. *In vivo* studies (Section 5) should be conducted using OECD TG 427 (OECD 2004a) or a similar protocol. Section 10.6 provides guidance on evaluating other study types, including ADME and human *in vivo* studies:

- As most substances have a higher permeability through rat (or rabbit) skin than through human skin, an appropriately conducted *in vivo* study is unlikely to underestimate dermal absorption in humans.

8. The term 'Triple Pack' refers to the three types of dermal absorption study: 1) *in vivo* animal; 2) *in vitro* animal; and 3) *in vitro* human (Section 6). The combined use of data from the three studies and two testing systems offers the potential for greater accuracy in estimating human dermal absorption because it corrects for the generally higher permeability of animal skin compared to human skin. Application of the data to refine dermal absorption values can vary between regulatory authorities:

- The 'Triple Pack' approach should be used to estimate a dermal absorption value only when the three studies are conducted under the same experimental conditions.

- In general, comparison of *in vitro* results using percentage absorption is preferred for finite dose application, rather than flux.

9. When considering the fate of the chemical remaining in the skin at the end of a study, the existing guidance should be consulted, in particular OECD GD 28 (OECD 2004c):

- The current default approach taken by nearly all regulatory agencies is to determine the dermal absorption value by adding the absorbed dose and the chemical remaining in the skin, following washing. This is appropriate for both *in vivo* and *in vitro* studies, unless compelling evidence is presented that demonstrates that some portion of the residue in the skin is unlikely to be absorbed.

- Section 7.1 should be read for guidance to assist in the consideration of whether to exclude some portion of the residue in the skin.

10. The current test guidelines recommend that the test preparations should be the same or a realistic surrogate to those which humans may be exposed to:

- Data generated on the test substance in a preparation other than the commercial formulation should be used only when the test preparation used in the study is very closely related to the commercial formulation in terms of solvent, surfactant content, skin irritancy and concentration of ingredients.

- Co-formulants in the test preparation may have a significant impact on absorption, and the outcome of a study in terms of flux or percentage absorption of the applied dose may be different when another vehicle is used. Section 7.2 contains a table of solvents and co-formulants known to affect dermal absorption.

- Because of physicochemical considerations, it may be assumed that skin penetration of water-based plant protection or biocide formulations and of solid materials (such as granules) will not be higher than for organic solvent-based formulations of the same active compound at the same concentration level, although there may be exceptions (see Section 7.2.2. on influence of formulation, paragraph 88).

11. The anatomical location of exposure affects the dermal absorption:

- Common exposure locations include abdominal or breast skin (human *in vitro*) or the forearm or back (human *in vivo*), but other anatomical locations demonstrate greater (or lower) absorption. The forearms and hands are potentially the areas most exposed to chemicals during occupational use. Nevertheless dermal absorption data obtained using abdominal and breast skin is considered representative of typical exposure situations. Studies with pesticides have indicated that dermal absorption may be greater than that for hands and forearms (Maibach and Feldmann, 1967; Maibach, 1971).

- For some non-occupational uses of chemicals, such as topically applied insecticides or cosmetics, which may involve application to other parts of the body, the anatomical location used in the exposure study should be taken into account. A good discussion of differences between anatomical locations can be found in EHC 235 (WHO 2006).

Part 2: Estimation of dermal absorption in the absence of specific studies

12. In some cases, specific experimental data on dermal absorption are not available. Under such circumstances, default values (Section 9) or alternative approaches to predict dermal absorption (Section 10) can be used:

- In the absence of data, 100% dermal absorption has to be assumed to cover a ‘worst case’ scenario.

- Many regulatory authorities will consider a reduction of the 100% default value to 10% if both the molecular weight is greater than 500 and the $\log P_{ow}$ is either below –1 or above 4.

13. Other approaches are available to estimate dermal absorption in the absence of data. These other approaches are outlined in Section 10 and have limited acceptability. They should be used only as a last resort as they provide only crude estimates of dermal absorption. If approaches outlined in Section 10 are used, the caveats described should be considered carefully, particularly if evaluating exposures to compounds in formulations and mixtures:

- ‘Read-across’ is applicable only to chemicals that have been demonstrated to be very similar in their chemical structure and physicochemical properties. Recently, the OECD has published general guidance on read-across and the formation of chemical categories (OECD 2007a).

- Modelling/QSAR is currently of limited applicability – the 'training set' must contain a reasonable number of closely related compounds. Even then, the formulated product may contain several adjuvants and the interaction between components makes prediction *in silico* unreliable.

- Studies used to evaluate ADME using the dermal route (*e.g.* using OECD TG 417; see OECD 2010) may be used to provide an idea of the magnitude of dermal absorption and a conservative rough estimate may be made. However, the dose and the vehicule used may not be relevant to field exposures.

- Data from oral and dermal acute toxicity studies should not be used. Data from repeat dose oral studies (with oral absorption data) and dermal studies should be used only where there is close similarity between the two studies in terms of design and effects seen.

PART 1: INTERPRETATION OF DERMAL ABSORPTION STUDIES

3. INTRODUCTION – TYPES OF DATA

14. Exposure to chemicals can occur, amongst others, through the oral, inhalation and dermal routes. In occupational settings, it is the inhalation and dermal routes that are the major routes of exposure. Occupational exposure to chemicals by inhalation has decreased to some extent, partly due to improved technology to minimise the exposure. Consequently, the dermal route is considered to be the primary route of exposure for occupational exposure to pesticides and industrial chemicals and for exposure to cosmetics.

15. Ethical considerations have led to it becoming increasingly difficult to conduct dermal absorption studies in humans. Therefore, risk assessors generally have to rely upon dermal absorption studies conducted in animals or from studies using human or animal skin *in vitro*.

16. Most toxicity studies are conducted via the oral route, and a limited number of studies are conducted via the dermal and inhalation routes. For example, a pesticide for food use registration conditionally requires (by US EPA: CFR 40 Part 158.500) only a 21-28 day dermal toxicity study, and a 28-day inhalation toxicity study if exposure via the inhalation route is of concern for the risk assessment. Often, the dermal toxicity studies are not suitable for risk assessment because the endpoints of concern observed in oral studies are not evaluated in a dermal toxicity study and because of some of the limitations of conducting the dermal toxicity study (for example, high dermal doses may lead to the ‘layering effect’).

17. As a result of these issues, it is necessary to use oral studies as a basis for estimating the risk of exposure via dermal and inhalation routes. In order to conduct the route-to-route extrapolation, it is important to know the dermal absorption of a chemical to estimate the internal dose. There are good discussions on this subject available in the literature (for example, WHO 2006; EC 2004).

18. Dermal absorption studies are conducted using *in vivo* methods (US EPA 870.7600, OECD TG 427) and *in vitro* methods (OECD TG 428). With respect to pesticides, the results of the *in vitro* dermal absorption studies alone may be accepted for risk assessment purposes in the European Union and other countries; however, NAFTA countries (the USA, Canada and Mexico) do not currently accept the results of *in vitro* studies alone for risk assessment purposes (NAFTA Dermal Absorption Group Position Paper On Use of *in vitro* Dermal Absorption Data in Risk Assessment, 2008). Although this statement may apply to pesticides and biocides, it does not necessarily apply to other types of commercial products regulated in the United States. This is discussed further in Section 4.

19. In the absence of dermal absorption studies, risk assessors will need to estimate dermal absorption using default assumptions and other considerations. The methods for estimating dermal absorption in the absence of specific studies are discussed in Part 2 of the document.

4. *IN VITRO* DATA

4.1 Introduction

20. For the determination of dermal absorption values of chemicals for regulatory purposes, *in vitro* studies can generally be used in one of the following two ways:[1]

- To compare the permeability of human and rat skin either in the same or in two separate studies with a comparable design using finite doses. The resulting ratio can then be used to correct or adjust the percentage of dermal absorption obtained in the rat *in vivo* (Section 5) provided the test preparation was the same, and the applied concentrations were at least similar (i.e. the 'Triple Pack' approach, see Section 6).

- To use human skin *in vitro* as stand-alone data to predict the expected dermal absorption by humans under field conditions without further conversion or correction.

21. It is generally recognised that regulatory authorities around the world currently have differences in the acceptability and use of *in vitro* data. Depending on the views and decision practice of the responsible national authorities, the use of the *in vitro* study on human skin may be considered as a basis for establishing a dermal absorption value. During the European Union evaluation process for pesticides under Directive 91/414/EEC, the dermal absorption values of many pesticides have been estimated this way (e.g. EC 2003a; EC 2003b; EFSA 2006). However, in NAFTA countries (the USA, Canada and Mexico), the results of the *in vitro* dermal absorption studies alone are not acceptable for risk assessment purposes because it is considered that there is still too much uncertainty in results from differing protocols to use *in vitro* human data as a stand-alone source of information (NAFTA, 2009). Although this statement may apply to pesticides and biocides, it does not necessarily apply to other types of commercial products regulated in the United States and Canada. It is beyond the scope of these guidance notes to provide a harmonised regulatory or scientific view on the use of *in vitro* data for regulatory risk assessment. It should be noted that in the assessment of some other types of chemicals, such as cosmetics, dermal absorption data, where available, are mostly from *in vitro* studies alone.

4.2 Species selection

22. In most cases, *in vitro* studies on human skin or rat skin (or both) are available. If reliable studies on human skin are available, these should be given preference over studies performed on skin from other species. Rat, mouse and rabbit skin may be more permeable than human skin, but it is not necessarily always the case (Handbook of Pesticide Toxicology, 2001). Sometimes, studies on skin obtained from other species such as monkey or pig or on artificial and cultured skin (epidermis grown from keratinocytes) are submitted. Data show that rat, mouse and rabbit skin are generally more permeable than human skin, but that the use of monkey or pig skin may not always result in a conservative estimate (ECETOC 1993). Pig skin has been shown to be a good surrogate for human skin and is commonly used in the cosmetic industry for *in vitro* studies. However it is noted that there are issues of limited experience, technical problems and many uncertainties of the appropriateness of alternative species for testing of pesticides and biocides. Specific expertise will be needed to justify the choice of such a test system and for interpretation of the data. Accordingly, the use of *in vitro* studies from any other species than rat and human to provide an estimate of dermal absorption values for the risk assessment of pesticides and biocides is not generally supported at this time, unless sufficient justification can be provided.

[1] Other scientific objectives such as investigations on partitioning of substances to the different skin layers or skin metabolism are out of the scope of this document.

23. An isolated *in vitro* study on rat skin without additional data is unlikely to underestimate absorption, and it could provide a rough estimate to replace a worst-case default value (of mostly 100%) in risk assessment (see, for example, van Ravenzwaay and Leibold (2004), or EHC 235 (WHO 2006)).

24. Published data give an inconsistent picture, but the weight of evidence suggests that the predicted dermal absorption in humans will be overestimated in most cases when the estimation is based on an *in vitro* study on human skin if all of the test substance retained in the skin is included. See Annex III and EHC 235 (WHO 2006) for a review of the literature. It is generally acknowledged that a limitation of the predictive value of *in vitro* data has been their high variability, which has been demonstrated in an inter-laboratory comparison conducted in 2004 (van de Sandt et al. 2004); however, it can be expected that increasing standardisation of experimental conditions after the adoption of OECD TG 428 (OECD 2004b) will help to reduce this variability. . In assessment of other types of chemicals, such as cosmetics, dermal absorption data, where available, are mostly from *in vitro* studies.

25. Human skin for *in vitro* studies is either taken from autopsies (cadaver skin) or obtained during cosmetic surgery. Permeability of human skin can be very different, depending on the site of body surface from which the skin samples had been excised: for example, the forehead or scrotum are more permeable than the back, the abdomen, the thighs or the forearms. The evaluator should, therefore, always consider the source of the skin used in testing and the relevance to the exposure being assessed (see WHO 2006 for a comparison of absorption from different anatomical locations).

26. During occupational exposure, less permeable body regions, such as the forearm are likely to be exposed to a higher deposition of compounds and for a longer time interval. As these areas are more relevant for real-world conditions, the data can be used in risk assessment. Neither the sex nor the racial origin of the donors are considered to have a significant impact on dermal absorption (WHO 2006).

4.3 Skin samples to be used and details of the study design

27. For recently conducted studies, it can be expected, and it is generally required, that guideline OECD TG 428 (OECD 2004) has been followed. However, because that guideline was adopted only in 2004, many studies that are to be evaluated will not be in full compliance with it. This Guidance acknowledges that not all available dermal absorption studies will have been conducted in accordance with OECD test guidelines, and thus where studies have been conducted prior to 2004, or done outside the recommendations of the OECD TG's, the following guidance is provided to allow an evaluation of the reliability of a non-OECD guideline study.

28. Regulators will have to carefully check the acceptability of non-OECD compliant studies on a case-by-case basis by comparing the study design and the reporting quality with current requirements. Additionally, some countries have additional guidance for specific groups of chemicals, for example pesticides and biocides (EC, 2004) or cosmetics (EC, 2006).

28. Crucial points might be the clear description of skin origin and preparation and the proof of skin integrity prior to use by an appropriate method (see for example Davies *et al.* (2004)), temperature (preferably around 32°C), the choice of a suitable receptor fluid (in which the test compound must be adequately soluble, see Section 4.4), the description of the diffusion cells used, the actual area of skin dosed, the number of cells/samples and donors, the duration of study/sampling period (preferably not more than 24 hours for substances that penetrate the skin rapidly), and the determination of the amount retained in skin after washing, irrespective of whether tape stripping was performed or not.

29. If the amount of chemical remaining in the treated skin *in vitro* has not been analysed, the study will usually be considered as unacceptable.

30. *In vitro* methods are designed to measure the penetration of chemicals into the skin and their subsequent permeation across the skin into a fluid reservoir, as well as partition to the different skin layers and possible deposition therein. Provided the excised skin sample is intact and its integrity has been proven by appropriate methods, it can reasonably be assumed that its barrier function to what is generally a diffusional process has been maintained *in vitro* (also after frozen storage (Harrision et al., 1984, Bronaugh *et al*., 1986 and Steinlig *et al.*, 2001). Then, in principle, the mechanism of skin penetration may be regarded as the same as *in vivo*.

31. Accordingly, non-viable but intact skin can be used to investigate percutaneous absorption. In addition, fresh, metabolically active (viable) skin can be used, but it should be recognised that many enzymes present in the viable epidermis (e.g. P450 class) have little or no activity within a few hours following resection (see Wilkinson and Williams 2008)). However, the latter case also allows limited investigations on skin metabolism and its possible impact on the absorption process. Different skin preparation techniques can be used (OECD 2004b), including dermatomed (split-thickness, 200–400/500 µm) skin as well as (by heat or enzymatically) isolated epidermis (with *stratum corneum*) or, when justified, full-thickness skin (consisting of *stratum corneum*, epidermis and dermis, up to 1000 µm thick); however any calculated fluxes with full-thickness skin should not be used. Skin thickness may contribute considerably to variation in absorption, with thinner skin preparations having increased flux and thicker preparations having a higher proportion of chemical retained in the skin (Wilkinson *et al*. 2006). The impact of this variation is reduced if all or part of the chemical retained in the skin is included in the dermal absorption value.

32. Both static (preferably with continuous stirring of the receptor fluid) and flow-through diffusion cells can be used (for details, see EHC 235 (WHO 2006)). The choice of occlusion or non-occlusion will depend mainly on the properties of the test substance (for example, volatility) and sometimes also on the exposure scenario. Non-occlusion is more likely to mimic the majority of pesticide, cosmetic, and industrial chemical exposure scenarios. Soaking of clothing or contamination of skin under gloves is sometimes considered as a realistic scenario for pesticide operator exposure in the field. If this scenario is considered to be relevant it should be addressed in the regulatory risk assessment and not in the decision for not recommending non-occlusion as the default approach.

33. Mostly, a finite dose experiment will be conducted since it better reflects occupational exposure. For comparison with occupational exposure to chemicals, exposure time should be at least 6 to 10 hours before washing with a relevant cleaning agent to remove the non-absorbed material: this time is consistent with the duration of a normal working day. However, caution would be required when interpreting a study which was terminated at this stage. Instead, a sampling period of 24 hours is preferred as it allows the absorption process to be better characterised. That is, the experiment should be terminated 14-18 hours after the skin has been washed.

34. Studies with a total exposure period of 24 hours are also acceptable if the skin surface is washed at the termination of the study to remove the non-absorbed test material. This approach may be appropriate for certain exposure scenarios, for example ‘leave-on’ cosmetic or topically applied insecticide products, but it is likely to overestimate the exposure patterns for most agricultural or industrial uses of chemicals. To improve the understanding of the absorption process and to allow a precise calculation of the flux, frequent sampling of the receptor fluid should have been undertaken as outlined in OECD (2004c): a total of 6–12 sampling points over 24 hours. Sampling after 8 or 10 hours is of particular importance since this value might be used for refinement of the estimate.

35. A study duration of more than 24 hours should be considered with caution because skin tissue can be expected to deteriorate. Of course, for some substances, in particular those that are lipophilic, it may take longer for a chemical to migrate from a skin depot to the receptor fluid. From a regulatory point of

view, however, the resulting uncertainty can be readily overcome by including the amount found in the skin as potentially absorbed (see Section 7.1 for further discussion on assessing chemicals remaining in the skin).

36. The dermal absorption value can be calculated as a percentage of the applied dose by measuring the penetration of the test substance into the receptor fluid and the amount retained in the skin sample. Partitioning can be described in greater detail if the different skin layers have been analysed separately. In many studies, tape stripping is used to determine the percentage in the *stratum corneum*, although the number of tape strips can vary (sometimes up to 10 or 15). There is currently some international disagreement about whether or not part, or all, of the test substance retained in the *stratum corneum* should be included in the calculation. This subject is discussed in Section 7.1.

37. To increase confidence in *in vitro* results, some countries have suggested the presentation of data for reference compounds such a testosterone, caffeine, or benzoic acids that are obtained at the same laboratory at a time that was the same or close to the dates of the study under review, but it should be noted that OECD TG 428 does not require these reference compounds to be tested close to the study under review. Thus, preference should be given to studies conducted according to OECD 428 and Guidance Document 28 (as they meet the requirement of this paragraph) and in compliance with Good Laboratory Practices. Non GLP studies conducted according to OECD TG 428 should contain data on the absorption of reference doses.

4.4 Receptor fluid

38. The choice of receptor fluid is a very important factor while conducting *in vitro* dermal absorption studies, with the major consideration being that the receptor fluid should not act as a rate-limiting step in the permeation process due to the limited solubility of the test compound within the medium (see OECD GD 28 (OECD 2004c)). For example, a saline solution may be an appropriate receptor fluid for determining percutaneous absorption for hydrophilic compounds, but it is unlikely to be appropriate for lipophilic compounds.

39. A major and frequently mentioned obstacle is the difficulty of estimating dermal absorption of very lipophilic substances by *in vitro* methods. For example, Shah *et al.* (1989) reported the differences in percutaneous absorption of several pesticides using the static and flow through systems. Both *in vitro* methods significantly underestimated skin absorption of the highly lipophilic compounds chlordecone and hexachlorobiphenyl. Lipophilic substances are poorly soluble in most receptor fluids, and partitioning will be inhibited. *In vivo*, lipophilic compounds are readily taken up by blood once it enters the cutaneous capillaries. The receptor fluid used *in vitro* should serve the same role as blood does *in vivo*. However, unlike *in vivo* conditions, the receptor fluid volume may be more limited, particularly in static diffusion cells. The effect of this can be minimised by use of frequent sampling (and subsequent replacement with new receptor fluid, as should be done in studies of this type) or use of a flow-through system (USEPA 1992).

40. Studies on the penetration of the lipophilic chemical fluazifop-butyl through human epidermal membranes showed that *in vitro* skin penetration results using an aqueous ethanol receptor fluid predicted *in vivo* human results (Ramsey *et al.* 1994). However, *in vitro* receptor solutions consisting of tissue culture medium and polyethylene glycol (PEG) underestimated human *in vivo* absorption.

41. *In vitro* and *in vivo* percutaneous absorption through rat skin has been measured for cypermethrin (Scott and Ramsey 1987). Good agreement between absorption of cypermethrin through rat skin *in vivo* and *in vitro* was observed when the receptor contained 50% aqueous ethanol, 6% Volpo 20, or 20% calf serum.

42. Yang et al. (1989) compared the *in vivo* and *in vitro* percutaneous absorption of anthracene through rat skin. Volpo-20 (6%) was added to the receptor fluid to increase the percutaneous absorption of lipophilic compounds to mimic the *in vivo* absorption value.

43. Bronaugh and Stewart (1986) reported drastically low *in vitro* percutaneous absorption of DDT (1.8%) and benzo(a)pyrene (BaP; 3.7%) when the receptor fluid was normal saline. However, the *in vitro* percutaneous absorption was greatly enhanced for DDT (60.6%) and BaP (56%) when PEG-20 oleyl ether was added in the receptor fluid. Additionally, the *in vivo* percutaneous absorption of DDT and BaP was reported to be 69.5% for DDT and 48.3% BaP through rat skin, and the maximum absorption of cinnamyl anthranilate was achieved when 6% PEG-20 oleyl ether was added to receptor fluid for static systems and flow-through diffusion systems. Wester *et al.* (1985) reported a markedly different percutaneous absorption value for trichlocaban in human abdominal skin using a static and a flow-through system. The relative insolubility of this compound in aqueous receptor fluid may be responsible for the discrepancy between the results obtained in the static system (0.13-0.23%), flow-through system (6%), and *in vivo* absorption value (7%).

44. The results summarised above clearly indicate that normal saline may be adequate as a receptor fluid for hydrophilic compounds, but saline alone is likely to underestimate *in vitro* percutaneous absorption of lipophilic compounds. Compounds such as anionic surfactants or other solvents must be added to the receptor fluid in order to increase the uptake of lipophilic compounds. The addition of surfactants to the receptor fluid may alter the permeability characteristics of the skin (Riley and Kemppainen 1985), and skin integrity should be measured when such substances are added to the receptor fluid.

45. For lipophilic compounds, the receptor fluid may contain solvent mixtures such as ethanol and water (50% aqueous ethanol), <6% polyoxyethelene (20) oleyl ether in water, or 5% bovine serum albumin (Sartorelli *et al.* 2000; Bronaugh 2004).

5. *IN VIVO* DATA

46. The main advantage of *in vivo* data is that they are generated from a physiologically and metabolically intact system. As most substances have a higher permeability through rat (or rabbit) skin than through human skin, this approach is unlikely to underestimate dermal absorption in humans. The approach therefore provides an additional margin of safety. For further information and references see the WHO/IPCS Environmental Health Criteria 235: Dermal Absorption (WHO 2006).

47. The rat is the most commonly used species for animal *in vivo* studies, because it is widely used in other toxicity and toxicokinetic studies and the results are therefore directly comparable. Data from other species (monkey and pig) may be used as skin absorption properties have been shown to be more similar to those of humans than of the rat. These two species are comparatively difficult and expensive to maintain as test species, and there are ethical considerations for their uses.

48. There are several types of *in vivo* animal (rat) data that are useful for estimating the dermal absorption value. This section describes guideline dermal absorption studies, and other types of *in vivo* studies are discussed in Section 10.6.

49. Studies for *in vivo* dermal absorption produce the most comprehensive *in vivo* measurement of dermal absorption because the quantities of chemical and/or its metabolites are determined throughout the animal and in the excreta for an extended period. The guidelines require administration of the test substance in an appropriate test preparation and in dilution(s) at realistic dose levels.

50. *In vivo* studies can be conducted with or without radiolabelled chemicals. Additional challenges are present when unlabelled compounds are used and when extensive metabolism occurs without a clear biomarker being available. Concerns about metabolism of radiolabelled chemicals are limited to the positioning of the radiolabel on a potentially labile group. Further information and guidance on radiolabelling and metabolism should be sought elsewhere, as these technical issues fall outside the scope of this guidance.

51. The dermal absorption value (including or excluding the application site as appropriate) from the final time point is generally the most appropriate regulatory value for a study conducted according to OECD TG 427 (i.e. the value from the group sacrificed with the longest post-wash observation period). The value from the final time point should be compared with those at other time points to ensure that the selected value is consistent with the whole observation period. Note that if all animals are terminated at cessation of exposure (i.e. if there is no post-application observation period included) the whole amount in the application site skin after washing should be considered potentially absorbable (justification for the inclusion or exclusion of tape strips is discussed later in Section 7.1).

52. If another time point is to be used, then it should be clearly justified. For example, this may be appropriate if the final time point is clearly an outlier, or if the data have unusually high variability across the time points (as seen in the example in Annex II).

53. Where the duration of exposure is longer than what is expected in the field (for example, a 24 hour exposure before wash-off for an agricultural pesticide), then it may not be appropriate to use the value from the longest duration if this also represents an inappropriate exposure duration.

6. COMBINATION OF ANIMAL AND HUMAN *IN VITRO* AND HUMAN *IN VIVO* DATA

6.1 Introduction: the 'Triple Pack' approach

54. The term 'Triple Pack' refers to the combined use of three types of dermal absorption data from: 1) *in vivo* animal; 2) *in vitro* animal; and 3) *in vitro* human dermal absorption studies. The combined use of data from the three studies and two testing systems offers the potential for greater accuracy in estimating human dermal absorption because it corrects for rat skin generally having a higher permeability than human skin. It should be noted that the triple pack approach may not necessarily lead to a significant refinement of the values, and it will not necessarily provide a value lower than the human *in vitro* data alone. There is currently work ongoing to validate the "triple pack" approach for use in regulatory risk assessment (Ross *et al.* 2011; EFSA, 2010 (draft)).

55. Application of the data to refine dermal absorption values can vary between regulatory authorities. A refined dermal absorption estimate using data from the 'Triple Pack' may be derived using the following approach:

$$\textit{In vivo}\text{ human absorption} = \frac{(\textit{in vivo}\text{ rat absorption}) \times (\textit{in vitro}\text{ human absorption})}{(\textit{in vitro}\text{ rat absorption})}$$

The triple pack calculation leads to multiplication of variability/error propagation. Thus – while potentially improving accuracy – precision of the overall dermal absorption estimate is potentially reduced. At the same time, any "built-in conservatism from *in vivo* rat or *in vitro* results may be lost." and "The Triple Pack calculation is based on the assumptions that i) the factor between the dermal absorption *in vitro* and *in vivo* will be the same for rat and humans, and ii) the factor between absorption in rat and human skin will be the same in vitro and in vivo, despite the morphological species differences."

56. A second approach to use the Triple Pack dataset is to decide on the acceptability of *in vitro* human skin data. Whenever the *in vitro* data on rat skin is comparable to the results obtained *in vivo*, it may be concluded that the *in vitro* model produced acceptable figures for the particular substance or formulation. Under these circumstances, it is then concluded, that the data from *in vitro* studies on human skin will also be predictive for the human *in vivo* situation. Consequently, such data may be accepted for RA (but without correction for *in vitro/in vivo* differences based on studies with rat skin). For standardization, both the *in vitro* and *in vivo* study may be preferably performed at the same laboratory.

Example 1

57. The following hypothetical example demonstrates the approach using three studies that were conducted using the same experimental conditions (*e.g.* the same test preparation and dose per square cm):

in vivo rat skin: 35%

in vitro human skin: 7%

in vitro rat skin: 49%

in vivo human dermal absorption is estimated to be 5% using 'Triple Pack' approach because there is a 1:7 ratio in permeability between human and rat skin (35% x 7% / 49%)

58. Many competent authorities, mainly in Europe, currently apply the 'Triple Pack' approach described above. The position of the NAFTA countries (USA, Canada) regarding triple packs is outlined in the draft NAFTA Dermal Absorption Group Position Paper On Use of In Vitro Dermal Absorption Data in Risk Assessment (2008). As discussion on the use of triple packs in these countries is ongoing, further details regarding the NAFTA position will not be included in this guidance.

59. The 'Triple Pack' approach should be used to estimate a dermal absorption value only when the three studies are conducted under the same experimental conditions, including using identical concentrations of test substance applied per surface area, the same duration of exposure to skin, and the same test preparation (for example, formulations such as emulsifiable concentrates or granules or in-use spray dilutions).

60. The major disadvantage of the 'Triple Pack' approach is the ethical consideration of using large numbers of animals and the cost of conducting these studies. The use of 'Triple Pack' is recommended only when the data are already available and studies conducted under the same experimental conditions (see above paragraph 59) or it is absolutely necessary to refine the dermal absorption value due to concerns about high risk. In addition, some countries do not consider that *in vitro* studies are sufficiently validated for use as part of a 'Triple Pack' approach for human health risk assessment (see Section 4 for further discussion).

6.2 Use of the 'Triple Pack' approach in risk assessment

61. When valid (guideline-compliant and GLP or GLP-like) *in vitro* studies on human skin, *in vitro* studies in animals and *in vivo* animal studies are available and conducted under the same experimental conditions, then the 'Triple Pack' approach can be used to extrapolate the human dermal absorption values for risk assessment.

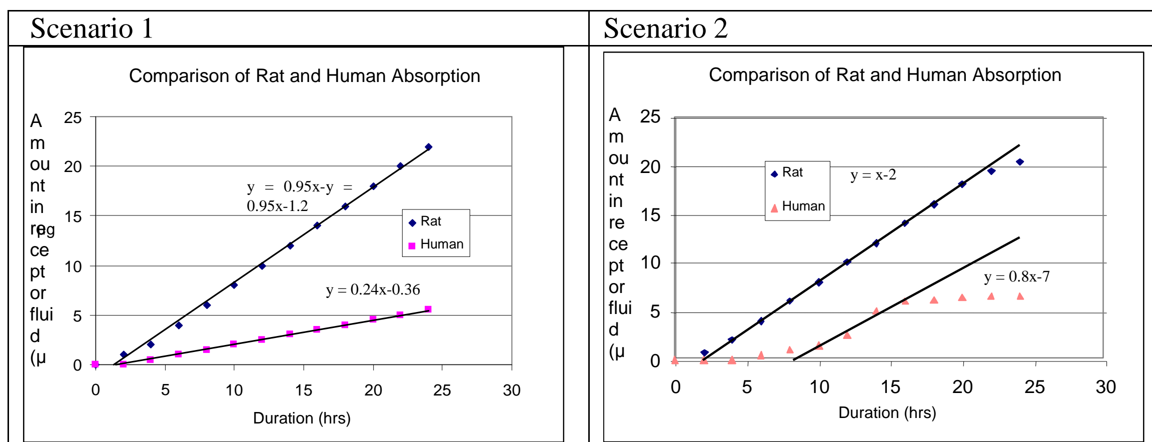
62. The question of whether to include skin-bound residues is addressed in Section 7.1. For *in vitro* studies, the OECD guideline (OECD 428) defines the 'absorbable dose' as '*that present on or in the skin following washing*'. A similar approach is recommended for the *in vivo* studies.

63. For comparison purposes – to establish an interspecies ratio for permeability through human skin versus rat skin – either the total absorption rate (in per cent) or the flux may be used. The flux describes the penetration of the substance per area unit (square centimetres, cm^2) and time (hours) and allows for semi-quantitative determination of species differences. However, the main disadvantage of using the flux is that the appearance of the test substance in the receptor fluid is the only relevant endpoint, and the amount deposited in the different skin layers is not considered. This results in a possible underestimation of the dermal absorption value when considering finite dose exposure. For example, where epidermal membranes are not used, there may be an underestimation of flux values as the result of retention of compounds in the skin, particularly in the case of lipophilic compounds. In general, the flux is not recommended for use in the risk assessment of pesticides, and the percentage absorption is preferred (see Section 10.3). However, if flux is used then the following should be considered:

64. The Permeability Coefficient (Kp) is a value, in units of centimetres per hour (cm/h) that represents the rate at which a chemical penetrates the skin. This is calculated from the flux divided by the applied concentration.

65. The linearity of the flux is dependent on a multiplicity of factors including species, skin thickness, receptor fluid, and formulation type. This is consistent with the statements of Bronaugh and Maibach (1987) and ECETOC (1993). If the duration of the linear phase of the flux is different between species, this can invalidate their use to calculate the inter-species ratio.

The following two stylised scenarios can demonstrate the difficulty of using flux:



The calculation of the ratio of absorption between the species can be summarised as:

Calculation of ratio	**Scenario 1**	**Scenario 2**
Flux based on slope of linear part of the absorption curve	(0.24/0.95) = 0.25	(0.8/1) = 0.8
Mass (µg) of applied dose absorbed during 24 hours	5.5/22 = 0.25	(6.5/20) =0.33
Impact on calculation of *in vivo* human	None	Flux calculations over estimate human *in vivo* absorption by 0.8/0.33 = 2.4

66. This comparison demonstrates that, in certain circumstances, the incorrect use of flux can overestimate *in vivo* human exposure. Differing absorption profiles require the use of percentage absorption at 24 hours to correct the *in vivo* rat absorption value. A third scenario could be included where human and animal skin show the same total permeated dose at the end of the experiment (24 h), but with higher flux over shorter time in animal skin (as the result of dose removal after 8 h and low depot effect) and lower flux in human skin that is maintained for a longer time (as the result of larger depot in thicker stratum corneum). This scenario shows that flux based calculations may as well underestimate absorption.

7. General considerations for the evaluation of dermal absorption studies

7.1 Chemical remaining in the skin

7.1.1 Definitions and existing guidance

67. The existing OECD test guidelines and guidance documents (OECD 2004a,b,c) form the basis of current considerations on whether to include or exclude the ‘absorbable dose’, which represents the test substance present in or on the skin following washing.

68. The current approach taken by nearly all regulatory agencies is to determine the dermal absorption value by adding the absorbed dose and the chemical remaining in the application site and surrounding skin following washing. This is appropriate for both *in vivo* and *in vitro* studies, unless compelling evidence is presented that demonstrates that at least some portion of the residue in the skin is unlikely to be absorbed. However, there is currently some international disagreement about whether part or all of the test substance should be included in the dermal absorption value that is retained in the *stratum corneum* and can be removed by tape stripping.

69. For *in vivo* studies, it is widely accepted that, if absorption can be demonstrated as complete (see 7.1.3) then all or part of the chemical remaining in the skin may be considered as unavailable for absorption.

70. For *in vitro* studies some regulatory authorities have a similar approach as for *in vivo* studies in that some of the amount retained in the skin may be considered as unavailable for systemic absorption. Others would include all of the test substance retained in the skin following *in vitro* exposure.

71. The following sections provide guidance to assist in the consideration of whether to exclude certain portion of the residue in the skin.

7.1.2 Tape stripping

72. OECD GD 28 states that skin fractionation may be conducted following exposure either *in vitro* or *in vivo*, noting that tape stripping can be difficult *in vitro* with epidermal membranes, rodent skin, study durations of more than 24 hours, or where the test preparation alters the *stratum corneum*.

73. Test substance retained in the top few layers of the *stratum corneum* (i.e. contained in the first few tape strips) may be removed by desquamation and therefore may not be absorbed. This includes substances retained in the top few layers of the *stratum corneum* as well as material that has not penetrated into the *stratum corneum* but is protected from wash-off, for example in hair follicles or sweat ducts.

74. In the European Union and some other countries, it is the practice at least for plant protection products (PPP) to exclude the amount that was found in the first (upper) two tape strips at study completion both *in vitro* and *in vivo*. It is important to address the impact of the use of certain materials for tape stripping (i.e. 'super glue'-based) on the acceptability of the results of tape stripping *e.g.* the current EFSA PPR Panel guidance draft states that "[...] glued (e.g. cyanoacrylate superglue) tape strips should not be used" (or else the complete tape strip fraction must be considered absorbed).

75. Test substance in lower layers of the *stratum corneum* may penetrate into the epidermis and further into the dermis, or may be removed by desquamation, and determination of the potential bioavailability of this test substance should be made on a case-by-case basis.

76. Dermal absorption is primarily a diffusion-driven process, and therefore test substance in the lower layers of the *stratum corneum* should be assumed to form a reservoir that may become systemically available, unless it can be demonstrated *in vivo* that absorption is complete and this test substance will remain in the *stratum corneum* until exfoliated (see 7.1.3).

77. In many studies conducted to date, separate analysis of the individual tape strips for radioactivity has not been performed. Instead, all tape strips are pooled before measurement. In this case the whole amount in the *stratum corneum*, as well as all the material retained in deeper layers, is generally considered absorbable and should be included in the calculation of the dermal absorption value (unless it has been demonstrated that absorption is complete). This highlights the importance of conducting a separate analysis of each tape strip rather than pooling the strips. However, any such analysis should address potential confounding factors such as those described in EHC 235 (WHO 2006).

7.1.3 Completion of absorption in vivo

78. Following an *in vivo* animal study, the 'absorbable dose' represents the amount of chemical present on or in the skin following washing. The following examples are provided as guidance on whether to include or exclude this absorbable dose in the calculation of the dermal absorption value:

1. In cases where an *in vivo* study is terminated just after cessation of exposure, there is no chance to determine the fate of chemical remaining in the skin, and it should be assumed that the dose remaining at the application site, including all material in the *stratum corneum*, is available for absorption (where there is no detectable systemic absorption see point 3 below).

2. If during an *in vivo* animal study there is measurable ongoing depletion of the dose from the application site following washing and a corresponding increase in cumulative absorbed dose over time, the dose remaining at the application site, including all material in the *stratum*

corneum (perhaps excluding the upper two tape strips), is considered to be available for further skin absorption.

3. Where data show serial ‘non detects’ in excreta, then this indicates that chemical remaining in the skin at the application site (including the *stratum corneum*) may be unavailable for further absorption. This serial ‘non-detects’ approach is appropriate either in cases where there is no detectable systemic absorption (excreted or remaining in carcass), or in those cases where the limit of detection is small in comparison to the amount excreted following wash-off. This will need to be determined on a case-by-case basis taking into account factors such as the shape of the excretion curve (how rapidly excretion drops off).

4. An *in vivo* dermal absorption study can be considered to have demonstrated completion of absorption if 75%* of the material absorbed by the end of the study (material in excreta + exhaled gasses + the carcass excluding application site) is present in the excreta or systemic compartment before the mid-point of the study. In this case, the bioavailability of any material remaining at the application site may be considered to have a minimal impact on the overall conclusion for the percentage absorbed. All material remaining in the skin at the application site (including the *stratum corneum*) may be excluded from the amount absorbed.

* The reason for this approach is that 75% represents two half-times. This guidance assumes that if 75% of the absorption occurs within half of the study duration, the total study duration should cover four half-times. Four half-times will cover more than 93% of the potential absorption assuming normal (single) exponential conditions. This approach has not been validated with use of real-world data at the time of publication of this guidance document, and is based on the expert opinion of the EGDA.

Figure 1*: Examples of representative absorption (as a percentage of the total) vs time profiles*

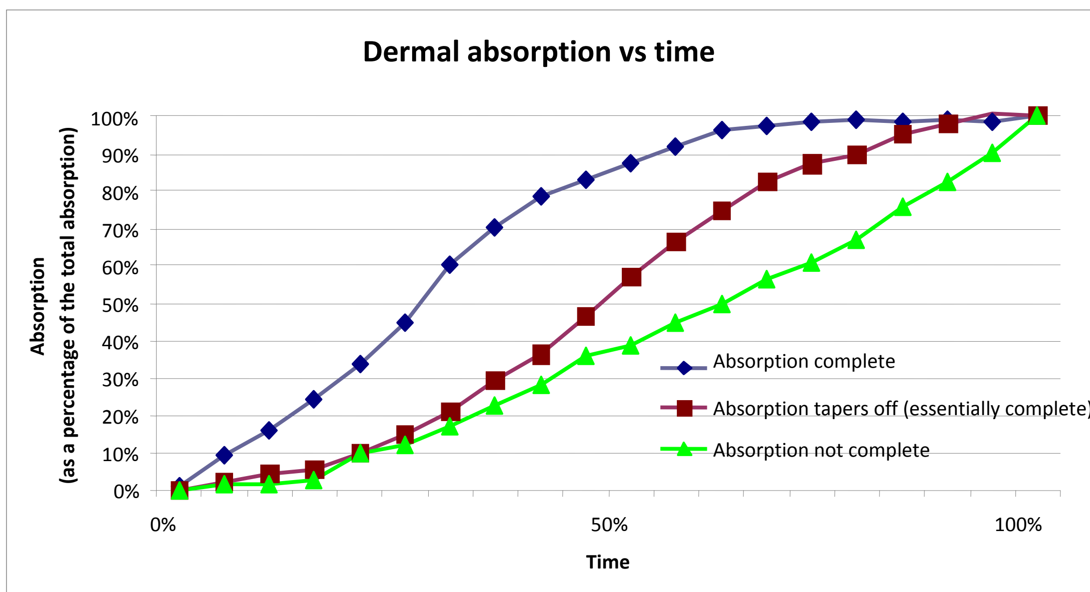
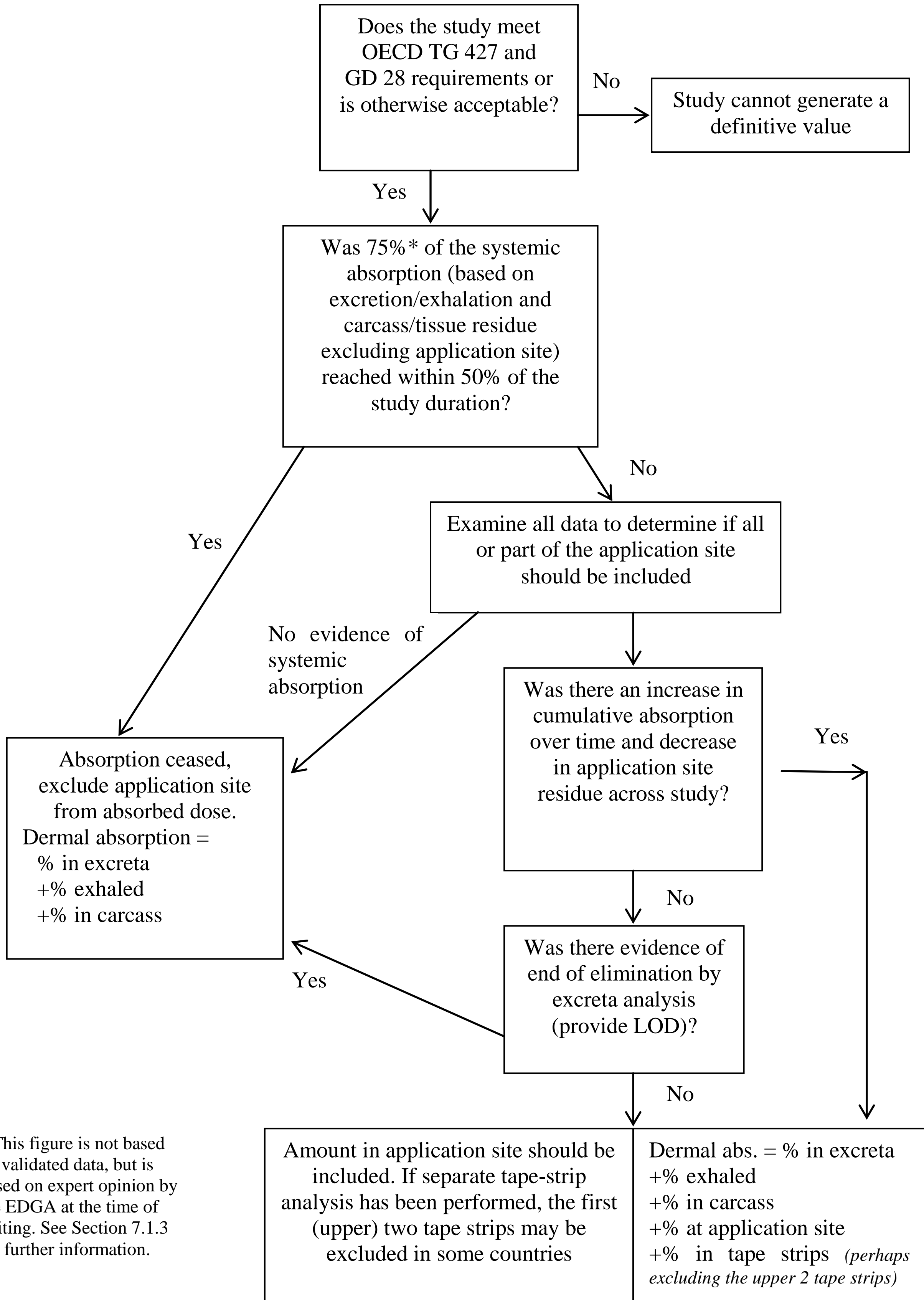


Figure 2. **Fate of chemicals remaining in application site skin *in vivo* – decision tree**



* This figure is not based on validated data, but is based on expert opinion by the EDGA at the time of writing. See Section 7.1.3 for further information.

7.2 Effect of formulation

7.2.1 Test preparations

79. Test preparations are either commercially available formulations (for example, cosmetics, plant protection or biocidal products and their field dilutions), or the test substance alone is applied in a suitable vehicle, which should closely match the proposed commercial formulation. In the latter case, expert judgement is warranted to determine whether the results can be used in risk assessment for a particular product containing this test substance. The reviewer must always be aware that co-formulants in the test preparation may have a significant impact on absorption and that the outcome of a study in terms of flux or percentage absorption of the applied dose may be different when another vehicle is used. See Table 1 for a list of solvents and co-formulants known to affect dermal absorption.

80. Usually, different concentrations (dilutions) are tested. These may include a concentrate or 'neat formulation' to mimic exposure (for example, upon mixing and loading a concentrate). At least one representative ready-to-use dilution may be used to mimic operator exposure when the chemical is handled or used in the field. It is common that the test substance is ^{14}C-radiolabelled, but non-radioactive material can be used if appropriate and if sufficiently sensitive analytical methods have been established.

7.2.2 Influence of formulation

81. Percutaneous absorption of chemicals from a specific vehicle depends on the partitioning of chemicals from the vehicle and solubility of a chemical in the vehicle. The influence of the vehicle on absorption has been well documented in the scientific literature. In addition the vehicle may change the integrity of the skin, and this influences absorption. Dimethyl sulfoxide (DMSO) is a polar solvent that has been intensively investigated. Stoughton and Fritsch (1964) found that penetration of hexopyrronium bromide (quaternary) and hydrocortisone was enhanced when they were applied in DMSO. Bronaugh and Franz (1986) compared percutaneous absorption of benzoic acid, caffeine, and testosterone in different vehicles through human skin using *in vivo* and *in vitro* methods. The authors reported that caffeine penetrated most readily from a petrolatum vehicle and the greatest testosterone absorption was from a water gel.

82. Small differences in the test preparation can greatly influence the *in vitro* penetration profile. However, as a general pragmatic rule, formulations can be considered similar when the content of each solvent/ surfactant/ detergent/ emulsifier is within 25% of the actual concentration of the tested formulation. Further, partitioning can be enhanced or evaporation of the vehicle may impede penetration, with white-spirit based test preparation having greater effects than acetone (Dick et al. 1997). Griffin *et al.* (1999) reported that the skin penetration of chlorpyrifos (as estimated from the amount recovered in receptor fluid) was about 1.5 times greater for a commercial concentrate vehicle than for an ethanol vehicle. Additional co-formulants such as stabilisers, safeners but also adhesives or antifreezing agents might alter physical or chemical properties of the preparation.

83. Regulatory authorities have recognised the influence of the vehicle on dermal absorption. The EPA-870.7600 test methods for dermal penetration (USEPA 1996) recommend that the vehicle system used should duplicate that under which field exposure occurs. Likewise, OECD TG 427 and TG 428 (OECD 2004a and 2004b) also recommend conducting tests using test preparations that are the same (or a realistic surrogate) to those that humans may be exposed to.

84. Formulations may range from a simple granule to complex multiphase solution and the potential exists for the physical form or the presence of differing additives and adjuvants to impact on the absorption

characteristics of the test substance. Further, products may be formulated to contain nanomaterials. The effects of nanotechnology have not been addressed in this guidance.

85. In addition to the specific chemicals present in a formulation, pH is also an important consideration because their state of ionisation at physiological pH (*e.g.* skin) or at the pH of the formulation will affect the overall net charge, which influences the ability to cross hydrophobic membrane barriers such as skin. The pH may also affect the irritant properties of the formulation and impact on the dermal absorption in this way.

86. In general, the dermal absorption value following finite exposure to a test substance in a highly diluted product (as measured in valid experiments) could be used to estimate skin penetration of a formulation that is of the same composition but less diluted because, in many cases, the percentage dermal absorption from a less concentrated product is higher and thus provides a conservative estimate for a more concentrated product. However, an estimate from a lower concentration may not result in a conservative estimate for skin-irritating or volatile substances (Buist *et al.* 2009) or where the values have been obtained from formulations which differ significantly. A flowchart to assist with estimating dermal absorption using data on different formulations/dilutions is provided in figure 3.

87. Table 1 lists some solvents that have been shown to increase the penetration of certain chemicals. Care should be taken when a chemical is presented in a new formulation that contains these solvents, and this may be a case where *in vitro* studies are particularly useful to bridge across formulations. However, it should be noted that the effect of any particular solvent on any particular chemical could not be easily predicted, with many differences not easily explained by a simple classification into hydrophilic or hydrophobic chemicals.

Figure 3. Flowchart – estimating dermal absorption using data on different formulations/dilutions

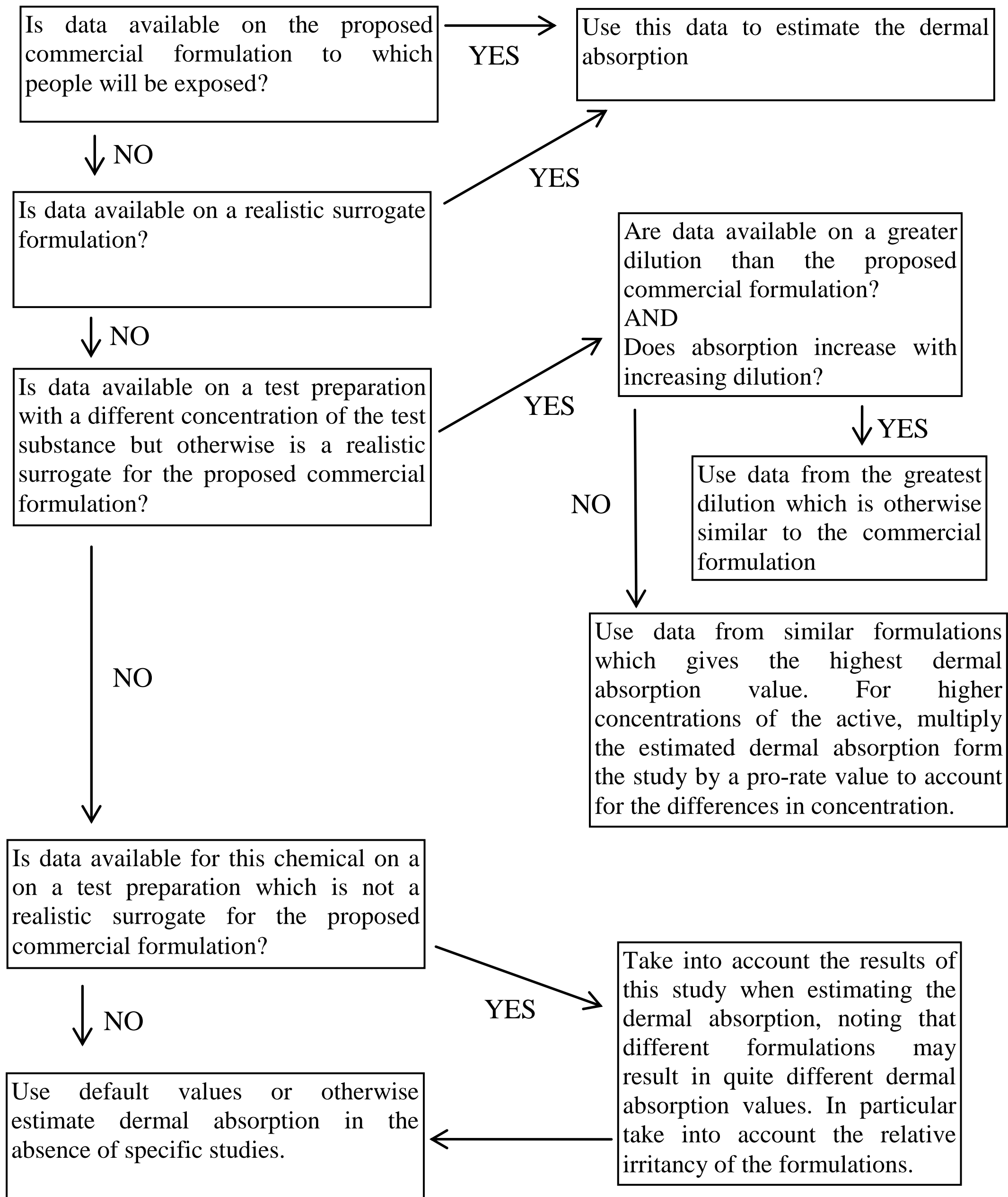


Table 1. Effects of some solvents or co-formulants on dermal absorption

Component	Mechanism	Notes	Effect	References
Mineral oils and co-solvents	Increase permeant solubility in vehicle		Increase solubility of lipophilic permeant in vehicle, can reduce thermodynamic activity and skin permeation of lipophilic permeants	Bronaugh and Franz (1986)
Dimethyl sulfoxide (DMSO), dimethylformamide (DMF), decylmethyl sulfoxide (DCMS)	Aprotic solvents—alter keratin and bilayer lipids	Effect is concentration dependent	High concentration causes increased penetration of hydrophilic and lipophilic permeants and also skin irritation and damage (erythema and wheals). 15-fold increase in caffeine penetration reported with DMF. Effect of DMSO on animal skin *versus* human membranes varies, with rodent skin permeability increasing substantially more than human	Maibach and Feldmann (1967), Southwell and Barry (1983), Notman et al. (2006), Al-Saidan et al. (1987)
Fatty acids, *e.g.* lauric acid, oleic acid	Alter bilayer lipids	Effective at low concentrations of less than 10%, particularly with propylene glycol	Enhancement greater with hydrophilic than lipophilic permeants examples: Oleic acid: 28-fold increased flux; salicylic acid: 56-fold increased flux; 5-flurouracil: 10-fold increase	Cooper (1984), Goodman & Barry (1988), Goodman & Barry (1989)
Pyrrolidones e.g. N-methyl-2-pyrrolidone (NMP)	Aprotic solvent—enhance solubility in *stratum corneum*		Enhancement greater with hydrophilic than lipophilic chemicals May cause irritation and erythema	Sasaki et al. (1991), Barry (1987), Williams & Barry (2004), Williams (2003)

Component	Mechanism	Notes	Effect	References
Dermal irritants, including urea	Hydrotrope and keratolytic. Vasodilation / enhanced blood flow and modulation of skin lipid fluidity.		Vasolidation and inflammation cause increased cutaneous blood flow with effects on the distribution of the substance. Corresponding changes in skin temperature enhance lipid fluidity, increasing substance solubility in the SC.	Barry & Williams, 2004 Veterinary Pharmacology and Therapeutics, Chapter 2 (Riviere and Papich, 2009)
Alcohols	Enhance solubility in vehicle and *stratum corneum*, lipid extraction on prolonged exposure	Ethanol is an enhancer at up to approx 60%; high concentrations cause dehydration and reduce permeation	Ethanol permeates skin rapidly; common solvent Nitroglycerin: 5- to 10-fold increased flux Estradiol: 10-fold increased flux	Kurihara-Bergstrom et al. (1990), Berner et al. (1989), Pershing et al. (1990)
Surfactants	Solubilise lipids in *stratum corneum*, interact with keratin		Increase TEWL *in vivo* Non-ionic surfactants (e.g. Tween) have minimal effect compared with ionic surfactants, e.g. SLS Note effect of surfactants on animal skin *versus* human membranes varies, with rodent skin permeability increasing substantially more than human May cause irritancy and erythema	Topker et al. (1990), Yu et al. (1988)

Component	Mechanism	Notes	Effect	References
Terpenes—components of essential oils	Increase solubility within *stratum corneum*, disrupt bilayer lipids		Substantial increase of hydrophilic but no increase of lipophilic permeants: - 34-fold increase 5-flurouracil (FU) by eucalyptus oil (human skin *in vitro*) - 95-fold increase 5FU by 1,8-cineole - No increase estradiol with 1,8-cineole - Synergistic effect between terpenes and propylene glycol.	Williams and Barry (1989), Williams and Barry (1991), Yamane et al. (1995), Cornwall and Barry (1994)

7.2.3 Solid vs. liquid formulations

88. In occupational situations, workers may be exposed to chemicals through different formulations such as emulsifiable concentrates, granules (witout solvent only), wettable powders, water insoluble powders, and adjuvants. Because of physicochemical considerations, it may be assumed that skin penetration of water-based plant protection or biocide formulations or of solid materials (such as granules) will be equal to or less than for organic solvent-based formulations of the same active compound at the same concentration level, although there may be exceptions. Provided there are no further co-formulants contained that might alter dermal uptake, experimental data obtained with an organic solvent-based test preparation may be considered as a 'worst case'. Accordingly, these study results could be rounded to the closest figure (such as 10% or 25%) to give an estimate for dermal absorption of the same or a comparable dilution of a water-based test preparation or of granules or powder.

7.3 Metabolism in the skin

89. Skin plays an important role in the metabolism of endogenous chemicals such as carbohydrates, lipids, proteins, and steroid hormones; and it plays an important role in the metabolism of exogenous compounds. The highest metabolising capability of the skin is observed in the epidermis layer of the skin and pilosebaceous glands. All of the major enzymes that are important for metabolism in the liver and other tissues have been identified in the skin (Pannatier *et al.* 1978).

90. On a body-weight basis, Phase I metabolism (such as oxidation, hydrolysis, reduction) in the skin is only a small fraction (2%) of that in the liver, but its importance should not be underestimated (Rice and Cohen 1995). Skin metabolism can be extensive because of the large surface area and volume of the skin. Mukhtar and Bickers (1981) reported that the activity of arylhydrocarbon hydroxylase (P450) activity in the skin exceeds 20% of that in the whole body when neonate rats were dermally treated with benzo(a)pyrene or Aroclor 1254.

91. The Phase II (such as conjugation, detoxification) metabolism capability in the skin has also been demonstrated. Mukhtar and Bickers (1981) reported that the glutathione S-transferase activity in skin cytosol was 15% of the corresponding hepatic activity in the neonatal rats dermally treated with benzo(a)pyrene or Aroclor 1254. For more detailed discussions on metabolic activity of the skin, see reviews by Kao and Carver (1990), Hotchkiss (1998), Hewitt et al. (2000), Bronaugh (2004a and 2004b) and WHO (2006).

92. Metabolism processes can certainly alter the *in vivo* absorption of a chemical through the skin. The influence of metabolism is much less significant for *in vitro* experiments due to lack of skin viability and reduced physiological functioning. Metabolism may not be an important consideration if the compound remains in the *stratum corneum*. However, it becomes an important factor for lipophilic compounds that cross the *stratum corneum*. Metabolism processes in the epidermis and upper dermis can make the lipophilic compound more hydrophilic and enhance the penetration of a chemical through the skin.

93. Where skin absorption data is used for risk assessment, metabolism is usually not a critical factor in interpreting the data. This is because the total percentage penetration of a compound is usually considered for the risk assessment. In addition, it is generally recommended that penetration studies be conducted with radiolabelled compounds to increase the sensitivity of the method used for absorption measurements. It is also recommended that the radio labelling position should be such that it is not easily labile and follows the major portion of the compound.

94. In summary, the skin is a vital organ of the body containing major Phase I and Phase II metabolising capabilities. Knowledge of the metabolism will enhance the interpretation of the dermal absorption study results. If the intent of the study is to determine the extent of absorption for the risk assessment then metabolism may not a critical factor. Likewise the capacity of reconstructed human skin models, such as EpiDerm FT model should be mentioned (Kang-Sickel *et al.*, 2010).

7.4 Mass balance

95. OECD TG 427 and 428 (OECD 2004a and 2004b) require a mean mass balance recovery of the test substance of between 90–110 % and the OECD GD28 (OECD 2004c) contains the same recommendation, with a caveat that for volatile test substances and unlabelled test substances, a range of 80–120% is acceptable. However, with the *in vivo* study design, recoveries outside this range may be acceptable but must be justified.

96. The criteria to justify mean mass balance recovery values outside the acceptance range can be summarised by the following examples:

1. Recovery values exceed the recommended range: If the recoveries exceed the accepted maximum range, the data generated should not be normalised because that would result in potentially underestimated absorption values**.** If these absorption values are not acceptable when a risk assessment is conducted, then the study should be repeated to address any bias resulting from excessive recoveries.

2. Recovery values below the recommended range: Low recoveries raise the concern that the value for absorbed dose could be lower than that which would be achieved from a study where the recoveries were within the guideline range. The reason for low recovery may be attributable to the following factors:

   - incomplete application of dose
   - loss to the experimental equipment
   - incomplete extraction from matrices (or incomplete collection of exhaled CO_2)
   - evaporation
   - unlabelled test preparations, metabolism or degradation.
   - Insufficiently high analytical LODs/LOQs, in particular where non-labelling analytical methods are applied.

104. If the results from some individual replicates (animals or *in vitro* test unit) show adequate recovery, then these can be compared with the low recovery replicates to see if the losses arise from absorbed or non-absorbed material.

105. The potential impact of low recoveries on the amount absorbed needs to be evaluated. In cases where the measured dermal absorption is low (e.g. less than 10%) then the low recovery may have a greater proportional impact on the value used for risk assessment. Inclusion or exclusion of the ‘missing’ percentage should be considered on a case-by-case basis in the context of the study (*i.e.* what was measured or collected and known limitations). For example, if the low recovery was due to incomplete

collection of exhaled CO_2, then correction for recovery could be performed by assuming that all the missing radioactivity could have been absorbed.

106. If the recovery rates are consistently below the recommended range across test animals, and the fate of the unrecovered material is unclear, there are two potential approaches to normalise the data, both of which could be applied on a case-by-case basis:

- One approach would be to normalise the measured dermal absorption value. For example, if the measured dermal absorption was 10% of the applied dose, and the recovery rate was 70%, then normalisation of the measured absorption (10%) by the recovery ratio (100/70) would obtain a new estimate of the dermal absorption (15%). This approach would be most appropriate where there is some indication that the low recovery is due to loss of unabsorbed material (for example by comparison with individual replicates which had adequate recovery).

- The other approach would be to include all the unrecovered material in the amount that is potentially absorbed. Using the example above, the measured absorption (10%) would be added to the unrecovered material (30%) to obtain an estimate of 40% absorption. For use in risk assessment, this value might be flagged as a worst-case assumption. This approach should be given the preference if the fate of the unrecovered material is unclear (appropriate worst case).

7.5 Use of mean or centiles; treatment of outliers and use of rounding

7.5.1 Introduction

107. Results of *in vivo* and *in vitro* dermal absorption studies can produce results that exhibit a degree of variability that is greater than that seen in many other types of studies used in human health risk assessments. This variability does not necessarily indicate poor experimental technique – it can be indicative of the physiological and biochemical inter-individual variability that exists in dermal absorption processes. There are several factors that might contribute to this, such as differences between donors of human skin samples, and slight damage to skin samples and application sites during preparation. When there is a high degree of variability, the appropriateness of using the mean value can be questioned, as could the overall ability to interpret the results in a consistent and meaningful manner. The guidance below is aimed at providing a simple pragmatic approach that takes into account the data values normally presented in dermal absorption studies conducted according to OECD TG 427 and 428 (OECD 2004a and 2004b).

7.5.2 Variability is relatively low

108. For values that are used to calculate the dermal absorption of an individual study where the standard deviation is less than 25% of the mean value, the mean value should be used.

7.5.3 Variability is high

109. If there is significant variation between replicates (the standard deviation is equal to or greater than 25% of the mean) consideration should be given to using a value other than the mean or possibly rejecting the study entirely. Consideration should be given to outliers, and in particular the relative distribution of the test substance through the skin. Where inter-individual variation is high (i.e. the standard deviation is equal to or greater than 25% of the mean) and group size is four or less ($n \leq 4$), the dermal absorption should be quoted as a range rather than an average, and consideration should be given to using the higher value in the range, rather than the average when conducting the risk assessment. For larger group sizes, the addition of a standard deviation to the mean value for absorption would give a value that

covered the upper 87th percentile value of the results, assuming a normal distribution (Chebyshev's theorem). Such an approach would be reasonably conservative and could reduce the need to repeat studies.

110. Where results with large variability are applicable to values used to compare relative absorption through rat and human skin in the 'Triple Pack' approach, a conservative method should be used. For example, if the rat value had high variability, then the mean value should be used in determining the ratio; if the variability was high for the human data, the standard deviation should be added to the mean value. However, it is important to avoid having to deal with such variable results, for example by standardising the procedure as much as possible, using more donors, etc.

7.5.4 Outliers

111. If any results are excluded as outliers (either in the preparation of the study report or by the regulatory evaluator), the reasons should be clearly stated in the study report and summary text. In addition, the full results from the samples considered to be outliers must be presented. It should be noted that consideration of results treated as outliers should include spuriously low values as well as high ones.

7.5.5 Rounding

112. To avoid a false impression of accuracy on the results of a study: dermal absorption values of more than 10% should be rounded to two significant figures, and values between 1% and below 10% should be rounded to one significant figure. There is currently some international disagreement on whether values of less than 1% should be rounded up to 1%. The rounding should be applied only to the final calculated value and not to intermediate values used in the calculation. For example, 8.7% becomes 9%; and 22.3 becomes 22%.

Example 3

113. The available dermal absorption studies of a hypothetical fungicide, all with low interindividual variability, were assessed with the following results:

rat *in vivo* study 0.3% including application site residue

rat *in vitro* study 1.2%

human *in vitro* study about 0.2%

114. The consistency of the data from this example indicate that dermal absorption is likely to be significantly less than 1% and rounding up to 1% is not justified. For this example, if the triple pack approach is followed, a value of 0.05% is obtained (1:6 ratio of human: rat), which would be rounded up to 0.1%. If the rat *in vivo* study were used in isolation then the value would be 0.3%.

7.6 The 'wash-in' effect

115. The 'wash-in' effect refers to the enhanced absorption that may occur by washing skin for cleansing purposes (Moody and Maibach 2006). This effect was reported for several pesticides in a series of literature reports involving *in vitro* tests with guinea pig, rat, human, and human tissue culture skin. For example, up to 32-fold enhanced penetration into the receptor solution of the insect repellent diethyl-*m*-toluamide (DEET) was reported for excised human skin following soap washing (Moody and Maibach, 2006). Particularly in cases where an *in vitro* test is terminated by a skin washing procedure (no post-wash sampling), consideration should be given to enhanced 'wash-in' absorption.

116. The mechanism(s) underlying the 'wash-in' effect is not fully understood, but it includes the effects of the washing method itself, such as those involving the type of soap or cleanser used, the duration and friction or pressure exerted on the skin surface, and possibly artefacts of *in vitro* methods, including those related to the skin specimen (such as animal species, anatomic site, skin hydration and pH) (Moody and Maibach, 2006).

117. As long as the skin depot is considered to be fully bioavailable, a 'wash-in' effect may not be as important. However, as 'wash-in' may lead to a rapid release or 'burst' of chemical to blood, the dermatotoxicokinetics should still be considered.

118. For *in vivo* studies conducted according to OECD TG 427 (OECD 2004a), the treated skin is washed with a cleaning agent at the end of the exposure period, and excreta are collected (usually for a number of days). Such protocols would include any chemical that has been made more bioavailable by washing, and indeed skin washing mimics "real world" exposure, where the skin is usually washed with soap at the end of the day. However, for protocols terminated by skin soap washing (*i.e.* where no sampling occurs after washing) the potential of enhanced absorption needs to be considered. For these protocols, it is prudent to include all chemical remaining in the skin as potentially absorbable. In all cases, irrespective of whether the study was conducted by *in vitro* or *in vivo* methods, the skin washing method needs to be fully described. The OECD Test Guidelines 427 and 428 (OECD 2004a,b) should also be followed to ensure the soap or other cleansing agent used is relevant to the exposure scenario being modelled.

PART 2: ESTIMATION OF DERMAL ABSORPTION IN THE ABSENCE OF SPECIFIC STUDIES

8. INTRODUCTION

119. If available, specific experimental data should be used to determine the dermal absorption value for a chemical in a particular test preparation. However, in many cases, such information does not exist, is not applicable (for example, because a certain formulation is not similar enough to the test preparation in the available studies), or cannot be used because of data protection. Under such circumstances, either default values must be used or alternative approaches to predict dermal absorption should be used. Some methods have been developed for this purpose and were found to be more or less useful for chemicals of interest. In contrast, their applicability to formulations (products with often more than one chemical of interest, and various co-formulants such as solvents and surfactants) is generally limited or unclear.

9. DEFAULT VALUES

120. As a first step, a default value should be used in exposure calculations. Usually, to cover a 'worst case' scenario, 100% dermal absorption has to be assumed. This conservative approach will result, in almost all cases, in a clear overestimation of actual absorption that provides a high level of protection. In practice, regulatory agencies will not ask for further data if, by calculating with the default value of 100% for dermal absorption, the intended use is considered as acceptable. The UK Chemicals Regulation Directorate has proposed in a draft guidance document (CRD, 2010), dermal absorption default values which are specific to plant protection products (PPP). Based on the analysis of available data on dermal absorption of PPP (investigation of 104 active substances discussed at EFSA PRAPeR meetings), it was concluded that a default dermal absorption value of 25% could be supported for the concentrated product. For dilutions, a default value of 75% might be applicable for the majority of products.

121. The physicochemical properties of a substance have a major impact on its dermal penetration. Thus, for example, it is widely assumed that for large molecules and those with either a very low or a very high octanol water partition coefficient ($logP_{ow}$), the skin is much less permeable than it would be for other, smaller molecules. Many authorities, particularly in Europe, consider this factor by reducing the 100% default value to only 10% if the molecular weight is greater than 500 and $logP_{ow}$ is either below –1 (that will suggest a high affinity for the aqueous phase) or above 4 (which is associated with high lipophilicity). This approach is explicitly mentioned in the European Union guidance document on dermal absorption (EU 2004; based on proposals by De Heer et al. 1999) and can be considered current practice in most European Union member states. However, the final scientific proof for neither this general assumption nor the arbitrary default numeric value of 10% has been provided. This is especially the case for chemicals in complex mixtures. Other countries may have different approaches and policies. In some countries the use of information on physicochemical properties to reduce a default dermal absorption factor should be corroborated with data from oral and dermal toxicity studies (Section 10.5).

122. Other physicochemical factors could also be important, but they have so far been used only occasionally (or not at all) for regulatory purposes. Examples of such factors include the physical state of the test preparation (for example, liquid, granules or powder), solubility in water and non-polar solvents, melting point, ionogenic state/pKa or dipole moment, lipophilicity, hydrogen bonding donor/acceptor potential, vapour pressure, surface tension, or corrosive properties due to extreme pH.

123. Further research is needed to establish clear relationships between physicochemical properties of test substances and dermal absorption. Such studies might open up new ways towards a more scientifically based choice of the appropriate default value in future.

124. Alikhan et al. (2009) tried to correlate dermal absorption in humans *in vivo*, based on urinary excretion, with physicochemical parameters such as molecular weight, melting point, water solubility or hydrogen bonding properties. The study examined a relatively small group of 12 pesticides from different classes (with some of them being no longer in use). Only a nearly significant inverse correlation between urinary excretion and molecular weight was found. In contrast, all the other physicochemical properties failed to provide any explanations for the extreme differences in dermal absorption, as indicated by the wide range (between 0.3% and 73.9% of the administered dose) that was found in urine. A similar picture was obtained for a group of 21 'miscellaneous organic compounds', and in that part of the study, molecular weight was not a predictor for dermal absorption. In contrast, for a chemically more homogenous group of 15 steroids, a possible impact of molecular weight, melting point and hydrogen bonding properties (in particular, the number of hydrogen acceptor groups on the molecule) could be shown. These results underline the importance of choosing the appropriate chemical domain (see also the Section 10.2 below).

125. In addition to the use of the 100% or 10% default values, it has been sometimes argued that dermal absorption cannot exceed the oral absorption rate. Accordingly, regulatory agencies might conclude that dermal absorption of a certain substance for which no experimental data are available can be assumed to have, for example, a maximum value of 60% if this percentage had been established for the oral absorption rate in an ADME study. Further work is required to scientifically validate this assumption. According to the European Union guidance document on dermal absorption, a direct comparison was confined to only 12 pesticides and the data had not been published (EU 2004). However, based on practical experience, it is very likely that it holds true for most substances despite the considerable differences in the absorption mechanisms from the gut and through the skin, but there may be exceptions, particularly for substances with very poor oral absorption. Furthermore, this practice would be applicable only for active compounds but not for formulations. The reasons are that there are usually no oral ADME studies for formulations, and the influence of co-formulants must not be ignored. For these reasons, estimates based on oral absorption are of limited value, not fully reliable, and therefore generally not recommended.

126. Kroes *et al.* (2007) studied the applicability of the 'threshold of toxicological concern' (TTC) concept to situations in which exposure is mainly by skin contact. For exposure calculations, they assumed a dermal penetration of not more than 8%, but this conclusion was based on practical experience with only 15 cosmetic ingredients. Accordingly, the database was small and the applicability domain very different to plant protection products in which, for example, solvents play a much bigger role, and for which much higher dermal absorption values frequently have been shown. This lower value is therefore unsuitable as a default value.

10. PREDICTION OF DERMAL ABSORPTION BY ALTERNATIVE APPROACHES

127. In some cases, default values are used in calculations, but the expected exposure is higher than the systemic toxicity threshold value. For example, if 100% dermal absorption is assumed, then the threshold value for operators, bystanders, residents, or workers may be exceeded for a certain product or application. In such cases, estimates can be refined by exploring further sources of information to estimate dermal absorption. Different approaches can be taken for this purpose, ranging from *in silico* predictions to a comparison of toxicity data from oral and dermal studies. They may be also combined to increase overall reliability.[2] A sound scientific basis for choosing a certain alternative method of prediction must always be

[2] It is noted that an increase in reliability can be expected only if methods are combined that are based on principles that are to some degree independent of each other.

provided. Companies should submit these 'theoretical considerations', and regulatory agencies should carefully check the validity of conclusions on a case-by-case basis because such efforts may save resources and reduce the need for animal testing. However, it must be emphasised that, at least for the time being, all these methods are mainly applicable to material in aqueous solution; their applicability to formulations is limited (a more detailed discussion of formulation effects is included in Section 7.2). These methods generally provide only a rough estimate of dermal absorption rather than precise values. Preferably, rounded values such as 10%, 25%, or 50% should be proposed to cover the high degree of uncertainty that is inherent to all approaches. For the same reason, companies should not expect that a proposal that was accepted by one regulatory authority will be necessarily considered sufficient by another.

128. In the following pages, the advantages and limitations of frequently used methods to estimate dermal absorption by theoretical considerations are briefly described.

10.1. Read-across

129. According to an OECD definition (OECD 2007a), the term 'read-across' describes a technique of filling data gaps. In the field of dermal absorption of chemicals, this approach has two main applications. The first one is to predict skin penetration of a test substance on the basis of experimental data obtained with a 'similar' compound, preferably from the same chemical group or class. The second application is to conclude from existing data for a certain test preparation to dermal absorption of a different preparation containing the same test substance (e.g. active ingredient). The first application is discussed below; the second application is discussed in Section 7.2.

130. If valid experimental data revealed a dermal absorption value of, for example, 6% for substance A, and if substance B, by expert judgement, is considered 'similar', a dermal absorption value of the same magnitude for both compounds can be reasonably expected (one-to-one or 'analogue' approach). On this basis, a regulatory agency might conclude that dermal absorption for substance B will not be higher than 10% and use this value for exposure calculations. This might happen if, for example, there was a reliable 'Triple Pack' for substance A that consists of *in vivo* and *in vitro* studies that were performed under GLP-like conditions and that are in compliance with OECD TG 427 and 428 (OECD, 2004a and 2004b).

131. The availability of dermal absorption data for different compounds from the same chemical class or group may enhance the confidence in this rough estimate (a many-to-one or 'category' approach). Thus, as a first step, companies should search for as many appropriate chemical 'analogues' as possible, and they should check whether those substances have been tested for dermal absorption. In many cases, it will be a practical obstacle that the validity of (mostly brief) information taken from large databases cannot be assessed. If there are no published data available, applicants will find it difficult to access dermal absorption studies that are the confidential property of other companies.

132. However, from a scientific and regulatory point of view, the main weakness of this approach is that 'similarity' is not clearly defined. It is obvious that two substances cannot be similar in absolute terms but only in their relationship to a given property. Physicochemical properties (such as the size of the molecule, $logP_{ow}$, melting point, ionogenic state) should be considered as well as chemical structures, and these properties might be of even higher importance. Recently, the OECD has published general guidance on read-across and the formation of chemical categories (OECD 2007a).

133. Additional uncertainty arises from the possible impact of different vehicles when data obtained with 'similar' substances are taken into consideration. Kroes et al. (2007) emphasised that dermal absorption of a test substance may differ substantially from formulation to formulation, depending on the

nature of the vehicle and the concentration of the test substance (a more detailed discussion of formulation effects is included in Section 7.2).

10.2. Quantitative structure-activity relationships (QSARs)

134. Quantitative structure-activity relationships (QSARs), also known as quantitative structure-permeability relationships (QSPeRs), have been frequently used to relate dermal absorption to various physicochemical descriptors and structural properties of the molecule. The aim is to predict dermal absorption of a chemical without a need for testing. QSARs provide statistically derived rules that have been developed on the basis of a so-called 'training set' and are applicable to a certain chemical space ('domain'). Recently, the OECD has developed criteria for validation of QSARs (OECD 2007b). A comprehensive overview on historical development and current use of QSARs and QSPeRs in the field of dermal absorption is given in EHC 235 (WHO 2006). There are a number of principal technical problems associated with modelling dermal absorption *in silico,* which have so far limited the applicability of QSARs to estimate dermal absorption. One of the biggest challenges is that penetration is influenced not only by molecular and physicochemical properties of the chemical itself but also by the properties of the vehicle and the structure and properties of skin, along with their interactions (Alikhan *et al.* 2009). Accordingly, Bouwman et al. (2008) reviewed 33 publicly available QSARs on skin absorption to assess their applicability in regulation. They found only one of them[3] suitable by giving reasonable predictions of skin absorption for 62 test compounds for which valid *in vitro* data were available. The authors concluded in 2008 that, 'none of the publicly available QSARs is suitable for general use in quantitative risk assessment'.

135. The only QSAR that is widely accepted in this field is the very simple one that takes into account molecular weight and $\log P_{ow}$ to support reduction of the 100% default value in some cases (see the above section on defaults). This approach should be further substantiated in the future.

136. The main difficulties that have prevented the applications of QSARs in the dermal absorption field so far include the following:

- The existing models have mostly been developed on small training sets of studies with substances that do not (sufficiently) cover the whole 'chemical space'. Thus, a few of the 'classical' *in silico* models (such as Potts and Guy 1992) are based on data of mostly hydrophilic compounds, and their predictive value ($r^2 = 0.67$ when applied to the 93 compounds of the so-called 'Flynn data set') may be lower if lipophilic compounds (such as most pesticides) are assessed. Thus, there is concern about the applicability of these QSARs to the domain under question.

- Even for chemical classes that have been part of the training set, the experimental conditions under which the results have been obtained (such as study design) are often not known. Taking into account the high variability in the conduct of dermal absorption studies (in particular before the OECD TG 427 and 428 came into force), it is questionable whether the studies were in fact comparable to each other and acceptable from a regulatory point of view.

- Most QSARs do not account for the dependency of dermal absorption on the concentration and dilution of the substance being assessed.

[3] According to Bouwman et al. (2008), this was the empirical model of Magnusson *et al.* (2004) that predicts flux in terms of molecular weight as the only descriptor by means of a comparatively large training set of up to 278 compounds, although some multiple entries were found.

137. For prediction purposes, QSARs should relate certain physicochemical properties of a test substance (for example, an active ingredient in a plant protection or biocide product) to a dermal absorption value that is to be expected. They do not account for the influences of the vehicle or co-formulants in the product.

138. Many QSARs in that field predict the dermal permeability co-efficient K_P or the flux but not the percentage of dermal absorption of a certain (finite) dose that was applied. However, this latter parameter has to be used in exposure calculations (see also below).

139. In spite of these problems, QSARs (perhaps in connection with read-across techniques) have the potential to become very useful in the prediction of dermal absorption. Future development will depend on the availability of large training sets from high-quality experimental dermal absorption data for as many chemicals with comprehensive physicochemical parameters and reliable information on vehicles, co-formulants, dilutions and study conditions as possible. On this basis, existing rules (such as those evaluated by Bouwman *et al.* 2008) could be rigorously scrutinised. New rules might be developed.

10.3. Use of flux or permeability coefficient K_P

140. Flux values are frequently reported, especially in the open literature, to describe dermal absorption under infinite dose testing conditions. However, this parameter is of limited value in evaluating risks arising from real-world exposure to finite amounts of dilute chemicals in a complex formulation.

141. The maximum flux at relevant exposure levels in milligrams per square centimetre per hour ($mg/cm^2/h$), calculated from the linear part of the absorption *versus* time curve, can be used for semi-quantitative comparison of absorption of chemicals between species, for example, as part of a 'Triple Pack', or to compare skin penetration of different compounds in the same species. As *in vitro* studies are used to determine flux, the same considerations should apply to evaluating these studies as described in the section on *in vitro* studies (Section 4).

142. The flux may also be applicable in situations where the exposure is similar to ‘infinite exposure’, such as exposure to chemicals in swimming pools or from ‘leave-on’ topically applied products. The use of steady-state flux could also provide a conservative estimate for short term exposure which may mimic an infinite dose, however this determination would be made on a case-by-case basis. There is no reliable procedure for calculating the amount or percentage of absorbed material from the flux for real-world finite exposure conditions. Furthermore, an apparent disadvantage of using flux is that the appearance of the test substance in the receptor fluid is the only relevant endpoint. The amount that is retained in the skin is disregarded but should generally be taken into account at least partly for risk assessment as potentially absorbed.

143. Many of the mathematical models that were designed to describe the process of dermal absorption (see section below) will result in predicting flux. The available model calculations (for example, Magnusson et al. 2004) will not provide an estimated value for the dermal absorption expressed as a percentage of an applied dose.

144. In an *in vitro* dermal absorption experiment under infinite-dose steady-state conditions, K_P is the steady state flux, divided by the difference in concentrations of donor and acceptor chamber (however, if the steady state is not reached within 24 hours, K_P can be derived from the non-linear part of the permeation kinetics).

145. The product of K_P and measured (or estimated) solubility in the same vehicle (usually water) provides an estimate of the maximum flux through the skin (Jones et al. 2004; Magnusson *et al.* 2004). However, in real-life occupational exposure to chemicals, such a scenario is rarely encountered, and so this

parameter is not suitable for a risk assessment that is based on dermal absorption of finite doses (Korinth *et al.* 2005). Again, even if K_P were applicable in a certain regulatory situation (such as cosmetics or pool chemicals), it will still be difficult to estimate a dermal absorption value from this parameter. Even so, the steady permeability coefficient or maximum flux has been used together with the lag time to describe non-steady-state or finite dose absorption (Roberts and Anissimov, 2005).

10.4. Mathematical models

146. A remarkable number of very different mathematical models have been developed to describe the process of percutaneous absorption and the partition of the absorbed material to the different skin layers and compartments either *in vitro* or *in vivo*. Comprehensive overviews were given by Fitzpatrick *et al.* (2004) and by Roberts and Anissimov (2005).

147. Mathematical models can be useful for better understanding of the skin penetration process and its details, particularly when physiologically based pharmacokinetic models are used (van der Merwe *et al.* 2006). Nonetheless, their relevance for prediction of dermal absorption in terms of an amount or a percentage of an applied dose appears rather limited.

148. For the time being, models are not considered to have been sufficiently validated for regulatory purposes. Possible influences of the vehicle or co-formulants will remain an obstacle that is hard to overcome. However, there are models that could be helpful to conclude from K_P data obtained under infinite dose conditions to estimates for dermal absorption after finite dose applications (WHO 2006). Certain models may also contribute to address specific issues such as the bioavailability of skin-bound residues in *in vivo* studies (Thongsinthusak *et al.* 1999).

10.5. Comparison of toxicity data from oral and dermal studies

149. Oral and dermal toxicity studies with repeated administration can be used to estimate dermal absorption of a substance provided the following apply:

- The species of animal used (mostly rat) and preferably the strain were the same in the studies, and the duration of the studies are similar.

- The range of parameters investigated is comparable and sufficiently extensive to detect target organ toxicity. Usually, clinical observations, monitoring of body weight and food consumption, haematology and clinical chemistry, gross pathology, organ weights and (any available) histopathology should be included.

- The number of animals per sex and group is sufficient for reliable statistical analysis.

- At least in the oral study, there should be systemic effects that prove internal exposure, although it is not mandatory that they be adverse (for example, a liver weight increase without histopathological findings or clinical chemistry changes may be sufficient for this purpose). However, meaningful comparison of the effects occurring after dermal administration is difficult where effects are C_{max}-driven . It is preferable that (similar) systemic effects be noted in the dermal study, too. Clear no observable effect levels (NOELs) and lowest observable effect levels (LOELs) should be established (rather than no observable adverse effect levels (NOAELs) or lowest observable adverse effect levels (LOAELs)). In cases where no adverse effects are seen in the dermal study, testing should be done up to the limit dose of 1000 mg/kg bw/day that will be considered the NOEL. Further, in comparison of the observed oral and dermal effects, it is considered appropriate to ignore local skin effects and only consider systemic observations.

- All available information on potentially relevant differences in toxicokinetic properties of the substance when administered by the two different routes, such as a significantly different metabolism, a different distribution or excretion profile should be carefully considered and a significant contribution of these factors can be ruled out.

150. When all these criteria are met, the NOELs and/or LOELs from the oral and dermal studies can be compared. The resulting ratios would suggest the magnitude of dermal absorption and could allow a rough estimate. For example: a NOEL of 20 mg/kg bw/day and a LOEL of 80 mg/kg bw/day in a 28-day oral study in rats might be compared to the limit dose of 1000 mg/kg bw/day in a 28-day dermal study in the same rat strain without any systemic effects occurring. The comparison of the oral LOEL to the dermal NOEL represents the worst case assumption, providing a ratio of 1:12.5, whereas comparison of the oral and dermal NOEL would give 1:50. It might be appropriate to make a conservative estimate of a dermal absorption value of not more than 10%.

151. Again, this approach will be usually be applicable only to the test substance in a simple test preparation because studies of these types are usually not available for formulations.

152. A general criticism of comparing the results of oral and dermal toxicity studies has been that, at dermal doses approaching the limit doses of approximately 1000 mg/kg bw/day, the depth of applied test material could be such that much of it was not in contact with the skin and not available for absorption. Such a situation would tend to compromise the reliability of the estimated systemic exposure, as opposed to the applied dose, in the dermal toxicity study.

153. It should be noted, that dermal absorption values derived from oral-dermal toxicity comparison will usually relate to a higher dose (e.g. the LOAEL). As shown elsewhere, low-to-high dose/concentration extrapolation may be acceptable (is considered usually conservative), while high-to-low dose/concentration extrapolation may lead to underestimation of dermal absorption (and is thus not recommended).

154. Other sources of uncertainty are the impact of enterohepatic circulation, and the possible impact of a first-pass effect following oral ingestion or, in certain cases, also dermal application. To account for that, target organs and toxic effects, if occurring, should be the same in oral and dermal studies.

155. Acute oral and dermal toxicity studies must not be used for comparison purposes because of the very limited range of parameters investigated and because of their frequent conduct as limit tests. Furthermore, in many cases, the different absorption kinetics would prevent meaningful comparison of the effects.

156. The use of maternal toxicity data from developmental toxicity studies is also not recommended because a direct comparison of effects in pregnant and non-pregnant animals should be avoided.

10.6. Other study types (including ADME and human *in vivo* data)

10.6.1. **In vivo** *ADME studies*

157. In rare cases, toxicokinetic studies (measuring absorption, distribution, metabolism, excretion and mass balance) have used the dermal route. The methodology for these studies is detailed in OECD TG 417: Toxicokinetics (OECD 2010). In principle, it is conceivable to compare results from such studies to those obtained after oral or intravenous administration. However, for a reliable determination of dermal absorption, it would be necessary to determine not only the plasma and blood level area under the curve, urinary recovery and mass balance, but also the amount retained in treated skin and its different layers following topical exposure. The recently updated OECD TG 417 recommends that an appropriate section

of treated skin should be analysed to determine residual substance. If more extensive data were available, the study could be considered a more extensive *in vivo* dermal absorption study and interpreted as such (see Section 5). If not, based on the internal dose, it would at best provide an idea of the magnitude of dermal absorption and a conservative rough estimate could be made. Care should also be taken that the exposure mimics 'in-use' exposure, including consideration, where relevant, of whether the test preparations mimic exposure to a concentrated product and a more diluted 'in-use' preparation.

10.6.2. The 'mass balance' approach

158. A mass balance approach refers to an experiment where the dermal absorption is inferred from the amount removed from the skin following the exposure period, together with urinary and faecal excretion data. In some cases plasma levels may also be available. This approach is often used for human studies and is sometimes seen in studies with laboratory animals.

159. This mass balance approach is problematic for chemicals with a relatively long half-life in the body. There can be significant undetected absorption of chemical remaining in the body at the end of the study, expired in air, or removed through normal skin cell turnover. Where possible, pharmacokinetics following dermal absorption should be compared with pharmacokinetics following intravenous dosing. Comparison of the plasma levels and excretion profiles for these studies can give a more accurate estimate of percentage dermal absorption. Factors that should be considered include:

- whether the sampling time is sufficiently long to allow nearly complete excretion – in cases where the sampling time is too short, the total excretion may be modelled (see for example Thongsinthusak *et al.* 1999)

- whether the chemical is excreted mainly in urine, faeces or expired air – a significant portion of the excreta may not be captured if only urine is sampled. The measured excretion should be adjusted to reflect the actual excretion based on pharmacokinetic data from laboratory animals

- whether the chemical is likely to accumulate in fatty tissues or undergo enterohepatic circulation – caution must be used for these chemicals, as either excretion data or plasma levels may significantly underestimate the absorption.

160. When radiolabelled test substances are not used, it is important to consider the applied dose and the limit of detection (LOD). The maximum amount of chemical excreted should be relatively large compared with the LOD. If this is not the case, then there may be significant undetected excretion, particularly for chemicals with an extended excretion profile.

10.6.3. Human **in vivo** *dermal absorption studies*

161. A well-conducted *in vivo* dermal absorption study in human volunteers would be the gold standard; however, such studies are unlikely to be available for most chemicals, and their use for regulatory purposes may not be allowed in certain countries (*e.g.* under the EC Regulation 1107/2009 concerning the placing of plant protection products on the European Union market, human tests shall not be performed). There is no OECD test guideline to describe how to conduct *in vivo* human dermal absorption studies. When evaluating these studies it may be instructive to refer to examples of human studies in the literature (see EHC 235 (WHO 2006) for a list of such studies).

162. The anatomical location of exposure is known to affect dermal absorption. The forearm or back is commonly used as the exposure area *in vivo* in humans, but other anatomical locations demonstrate greater (or lower) absorption. See EHC 235 (WHO 2006) for a review of the available literature. The forearms and

hands are potentially the areas most exposed to chemicals during occupational use. However non-occupational uses of some chemicals, such as topically applied insecticides or cosmetics, may involve application to other parts of the body. For example, the forehead has significantly higher absorption than the forearm (approximately 5-fold), and in cases where extended exposure to the forehead may occur, and where this is likely to be a major source of exposure, the increased permeability of the forehead should be taken into account when estimating the dermal absorption potential.

10.6.4. Human biomonitoring studies

163. Another approach that yields useful data is biomonitoring conducted on workers handling a chemical in the field. While this approach may be useful for risk assessment (see for example EFSA 2010), it is unlikely that the percentage dermal absorption can be extrapolated from such a study, as the exposure – the amount of chemical in contact with the skin – is not accurately known.

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ANNEX I

DEFINITIONS

The following definitions were taken from OECD TG 427 and 428 and OECD GD 28 (OECD 2004,a,b,c).

Absorbed dose: (*in vivo*) comprises that present in urine, cage wash, faeces, expired air (if measured), blood, tissues (if collected) and the remaining carcass, following removal of application site skin.

Absorbed dose: (*in vitro*) mass of test substance reaching the receptor fluid or systemic circulation within a specified period of time.

Absorbable dose: represents that present on or in the skin following washing.

Unabsorbed dose: represents that washed from the skin surface after exposure and any present on the nonocclusive cover, including any dose shown to volatilise from the skin during exposure.

Penetration enhancer: Adjuvant, which facilitates penetration of the test substance through skin.

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

Applied dose: mass of test preparation containing a specified mass of test substance applied per cm^2 of skin.

Exposure period: time from application of test preparation to removal at skin washing.

Dermal delivery: sum of the applied dose found in the treated skin and the absorbed dose at the end of the experiment.

Infinite dose: Amount of test preparation applied to the skin where a maximum absorption rate of the test substance (per unit area of skin) is achieved and maintained.

Finite dose: amount of test preparation applied to the skin where a maximum absorption rate of the test substance may be achieved for a certain time interval but is not maintained.

'In-use' preparation: the preparation of test substance that relates directly to potential human exposure (*e.g.* cosmetic or agrochemical formulations and dilutions thereof, a mixture of industrial chemicals in a solvent, etc.).

Flux: mass of test substance passing through a unit area of skin per unit of time under steady-state conditions in micrograms per square centimetre per hour ($\mu g/cm^2/h$).

Dislodgeable dose: mass of test substance that is removable from the application site.

Exposure period: time from application of test preparation to removal at skin washing.

Permeability coefficient (K_P): a value, in units of cm/h, which represents the rate at which a chemical penetrates the skin. This is calculated from the flux divided by the applied concentration.

ANNEX II

EXAMPLE EVALUATION

Study 1: *In vivo* dermal absorption of chem1 in the rat

Test material

^{14}C-chem1 (Pesticide Chemicals Corp, Batch No. 100-75, chemical purity 96.7%, specific activity 5.31 MBq/mg)

ProductA (Pesticide Chemicals Corp, Batch No. 081-7, Solution Concentrate containing 35% chem1). ProductA was tested neat and as a 1:1000 aqueous dilution to mimic the 'in-use' preparation.

Guidelines

OECD Guideline 427 (Skin Absorption: *in vivo* Method), 2004; EPA OPP 870.7600 (Dermal Penetration), 1996; fully GLP compliant.

Material and methods

Eight groups of male rats (4 rats/group) were exposed for eight hours to either A) neat Product A (5 mg chem1/cm^2) spiked with radiolabelled chem1 or B) 1:1000 aqueous dilution of Product A (0.005 mg chem1/cm^2) spiked with radiolabelled chem1. In both cases, the dosing volume was 10 µl/cm^2, and the treated area was about 10 cm^2. A semi-occlusive adhesive bandage was used to protect the test area. At the completion of the exposure, the skin was washed with 1% Tween 80. One group from each dose level was sacrificed immediately after this first wash. The other groups were sacrificed at 24, 72 and 168 hours, with a second skin wash before sacrifice. At termination, tape stripping was performed to remove the *stratum corneum*, with up to 20 tape strips taken until a 'shiny' appearance of the epidermis was observed.

Results

There were no clinical signs in the treated rats, and recovery was within the acceptable range. The distribution of radioactivity in the different compartments following application of neat Product A is shown in Table 1.

The dermal absorption value includes both the amount found in the systemic compartment (including excreta, blood and carcass) and the amount that is considered potentially absorbable (OECD 2004a, OECD 2004c). Excretion increased over time and was not complete at 168 hours, indicating ongoing absorption of chemical remaining in the skin into the systemic compartment. For the neat Product A, at the mid-point of the study (72 hours) the amount in the excreta and systemic compartment (0.471%) was significantly less than 75% of the amount at the end of the study (0.874%). Based on these findings, the

chemical remaining at the treatment site and surrounding skin was included when calculating the dermal absorption value.

However, it is considered that chemical remaining on the outer layers of the s*tratum corneum* would be removed through exfoliation, and is therefore unlikely to be absorbed. On this basis the first two tape strips were excluded. These tape strips contained around 30% of the radioactivity associated with the *stratum corneum*.

Due to the somewhat unusual variability seen across time points, particularly for the 1:1000 dilution, the maximum dermal absorption value was used, rather than the value from the final time point (it should be recognised that there is global debate regarding this determination, and it should be made on a case-by-case basis).

On this basis, the maximum estimated dermal absorption for neat Product A was 4.5%, based on the value at 24 hours, excluding the first two tape strips and including the remainder of the chemical retained in the skin.

For the 1:1000 aqueous dilution of Product A the maximum estimated dermal absorption was 12.8%, based on the value at 8 hours.

Table 1. Percentage absorption of radioactivity following application of neat Product A *in vivo*

Group (termination time)	Group 1 (8 hours)	Group 2 (24 hours)	Group 3 (72 hours)	Group 4 (168 hours)
SURFACE COMPARTMENT				
Skin swabs at 8 hrs	99.41 ± 1.16	94.12 ± 3.82	96.49 ± 3.37	93.00 ± 3.08
Skin swabs at termination		0.43 ± 0.22	0.40 ± 0.21	0.16 ± 0.06
Fur	-	-	-	2.17 ± 1.11
Dressing	0.94 ± 0.87	1.13 ± 1.00	1.64 ± 2.18	2.31 ± 1.52
Washed SKIN COMPARTMENT				
stratum corneum [a]	1.49 ± 0.45	3.20 ± 2.01	2.11 ± 0.59	1.00 ± 0.45
Tape strips 1 & 2	0.32 ± 0.12	1.59 ± 1.19	0.91 ± 0.44	0.57 ± 0.18
Treated skin [b]	0.38 ± 0.18	0.31 ± 0.27	0.10 ± 0.08	0.11 ± 0.04
Surrounding skin	0.25 ± 0.12	0.55 ± 0.57	0.35 ± 0.40	0.26 ± 0.22
Systemic COMPARTMENT				
Urine	n.d.	0.01± 0.04	0.03 ± 0.01	0.13 ± 0.05
Faeces	n.d.	n.d.	0.02 ± 0.02	0.24 ± 0.10
Cage wash	n.d.	0.01± 0.02	0.05 ± 0.01	0.04 ± 0.04
Cardiac blood	n.d.	0.003 ± 0.003	0.001 ± 0.002	0.004 ± 0.003
Non-treated skin	0.14 ± 0.05	0.10 ± 0.03	0.09 ± 0.03	0.15 ± 0.06
Carcass	0.28 ± 0.05	0.35 ± 0.04	0.28 ± 0.02	0.31 ± 0.03
Total % excreted and in systemic compartment	0.42 ± 0.05	0.473 ± 0.03	0.471 ± 0.02	0.874 ± 0.06
OVERALL ABSORPTION				
Total % directly absorbed	0.42 ± 0.07	0.46 ± 0.06	0.47 ± 0.08	0.87 ± 0.21
Total % potentially absorbable [c]	2.53 ± 0.55	**4.53 ± 2.25[d]**	3.02 ± 1.04	2.24 ± 0.90

a: Based on tape-strips excluding numbers 1 and 2, which were considered not potentially absorbable;

b: Remaining skin after tape stripping; c: Sum of systemic and washed skin compartments excluding tape strips 1 and 2; d: the maximum dermal absorption value was used rather than the value from the final timepoint (see text for further information); n.d. not detected; - no sample available.

Table 2. Percentage absorption of radioactivity following application of a 1:1000 dilution of ProductA *in vivo*

Group (termination time)	Group 1 (8 hours)	Group 2 (24 hours)	Group 3 (72 hours)	Group 4 (168 hours)
SURFACE COMPARTMENT				
Skin swabs at 8 hrs	68.55 ± 4.65	77.28 ± 9.68	78.81 ± 4.18	72.81 ± 6.66
Skin swabs at termination		2.51 ± 0.77	0.87 ± 0.49	0.33 ± 0.17
Fur	-	-	-	1.05 ± 0.45
Dressing	7.11 ± 8.01	5.87 ± 6.60	6.69 ± 4.22	4.52 ± 2.69
Washed SKIN COMPARTMENT				
stratum corneum [a]	4.12 ± 2.17	2.37 ± 1.33	1.48 ± 0.21	0.61 ± 0.31
Tape strips 1 & 2	1.09 ± 0.40	1.71 ± 0.82	0.49 ± 0.33	0.31 ± 0.23
Treated skin [b]	1.91 ± 0.21	0.59 ± 0.64	0.25 ± 0.14	0.16 ± 0.08
Surrounding skin	1.67 ± 0.97	0.32 ± 0.18	0.13 ± 0.03	0.33 ± 0.37
Systemic COMPARTMENT				
Urine	0.08 ± 0.03	0.54 ± 0.13	2.18 ± 0.15	3.32 ± 1.16
Faeces	0.02 ± 0.04	1.39 ± 0.24	4.05 ± 0.90	5.50 ± 2.33
Cage wash	0.04 ± 0.03	0.35 ± 0.10	0.39 ± 0.22	0.54 ± 0.29
Cardiac blood	0.03 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.01 ± 0.01
Non-treated skin	0.51 ± 0.13	0.29 ± 0.10	0.27 ± 0.06	0.22 ± 0.09
Carcass	4.45 ± 0.92	2.79 ± 0.76	2.17 ± 0.44	1.08 ± 0.29
Total % excreted and in systemic compartment	5.15 ± 0.95	5.40 ± 0.87	9.11 ± 0.98	10.67 ± 3.69
OVERALL ABSORPTION				
Total % directly absorbed	5.12 ± 0.99	5.40 ± 0.82	9.10 ± 1.28	10.66 ± 4.03
Total % potentially absorbable [c]	**12.81 ± 3.13** [d]	8.68 ± 2.64	10.96 ± 1.34	11.76 ± 4.63

a: Based on tape-strips excluding numbers 1 and 2, which were considered not potentially absorbable;

b: Remaining skin after tape stripping

c: Sum of systemic and washed skin compartments excluding tape strips 1 and 2

d: the maximum dermal absorption value was used rather than the value from the final timepoint (see text for further information)

- : no sample available

Conclusions

Following an 8-hour exposure *in vivo* to rats of either neat Product A or a 1:1000 aqueous dilution, the maximum estimated dermal absorption of chem1 was 4.5% and 12.8%, respectively. Due to the variability seen across time points, particularly for the 1:1000 dilution, the maximum dermal absorption value was used, rather than the value from the final time point.

Study 2: *In vitro* dermal absorption of chem1 in rat and human skin

Test material

^{14}C-chem1 (Pesticide Chemicals Corp, Batch No. 100-75, chemical purity 96.7%, specific activity 5.31 MBq/mg)

ProductA (Pesticide Chemicals Corp, Batch No. 081-7, Solution Concentrate containing 35% chem1). ProductA was tested neat and as a 1:1000 aqueous dilution to mimic the 'in-use' preparation.

Guidelines

OECD Guideline 428 (Skin Absorption: *in vitro* Method), 2004; fully GLP compliant.

Material and methods

Samples of shaved rat skin from the dorsal region and dermatomed human abdominal skin were exposed for eight hours to either neat Product A or a 1:1000 aqueous dilution of Product A as specified in Study 1. The integrity of the skin samples was assayed using trans-epidermal water loss.

Following addition of the test substance, samples of the receptor fluid were taken at various timepoints. At the completion of the 8-hour exposure, the skin was washed with 1% Tween 80. Another skin wash was conducted at 24 hours, after which tape stripping was performed and radioactivity measured.

Results

Recovery was within the expected range, and the test substance was adequately soluble in the receptor fluid (0.2 mg/mL). There was generally low absorption of the concentrate *in vitro* in both human and rat skin.

Chemical remaining on the outer layers of the s*tratum corneum* may be removed through exfoliation. On this basis the first two tape strips may be excluded. For human skin, these first two tape strips contained around 85% of the radioactivity associated with the *stratum corneum*, while for rat skin these strips contained around 40%. Due to this significant difference between rat and human skin the *in vitro* dermal absorption was calculated both with and without the first two tape strips.

Table 3. Percentage absorption of radioactivity *in vitro*

Dose level	Low dose (0.5 mg/mL)		High dose (500 mg/mL)	
Skin samples	Human (n=5)	Rat (n=5)	Human (n=5)	Rat (n=6)
SURFACE COMPARTMENT				
Skin swabs at 8 hrs	97.07	85.47	100.85	100.12
Skin swabs at 24 hrs	0.50	0.69	0.29	0.13
Material remaining in donor chamber	0.23	0.13	0.69	0.03
Total % non-absorbed	97.80	86.29	101.83	100.28
SKIN COMPARTMENT				
Skin [a]	0.21	0.71	0.04	0.77
Tape strips 1 & 2	1.17	2.33	0.71	0.73
stratum corneum [b]	0.34	3.16	0.05	1.25
Total % at dose site (including tape strips 1 & 2)	1.72	6.20	0.81	2.75
Total % at dose site (without tape strips 1 & 2)	0.55	3.87	0.10	2.02
RECEPTOR COMPARTMENT				
Receptor fluid (collected over 24 hrs)	1.19	8.25	< 0.01	0.07
Receptor fluid terminal	0.03	0.26	N.D.	< 0.01
Receptor chamber	N.D.	0.05	N.D.	N.D.
Total % directly absorbed [c]	1.23	8.56	< 0.01	0.08
OVERALL ABSORPTION				
Total % potentially absorbable [d] (including tape strips 1 & 2)	**2.95**	**14.76**	**0.82**	**2.83**
Total % potentially absorbable (without tape strips 1 & 2)	1.77	12.43	0.10	2.10
Total % recovery	100.73	101.05	102.63	103.10

n: number of skin cells used for calculation; a: amount remaining in skin after tape stripping procedure

b: tape-strips excluding numbers 1 and 2, which were considered by the notifier to be non-absorbed

c: including receptor fluid (0 to 24 h), receptor fluid at termination time and receptor chamber

d: sum of ‘Totals % directly absorbed’ and ‘Total % at dose site’

N.D.: not detected (below limit of detection)

Conclusions:

The potential dermal absorption was calculated both with and without inclusion of the first two tape strips. In the high dose study using human skin nearly all of the potentially absorbable material was found in the skin, and 93% of this was found in the first two tape strips. The use of the high dose human value without tape strips (0.1%) would result in an *in vitro* ratio of human: rat of around 20:1, which is inconsistent with the other results in this study (all around 4:1). On this basis, the conclusions below are based on the calculations in which the first two tape strips are considered potentially absorbable.

When the above approach is applied to this study, *in vitro* dermal absorption of chem1 in neat ProductA was 2.8% for rat skin and 0.8% for human skin (a ratio of 3.5:1). In the spray dilution the dermal absorption was 14.8% for rats and 3.0% for human skin (a ratio of 4.9:1).

Discussion of dermal absorption of chem1

The *in vivo* study on rats demonstrated dermal absorption values of 4.5% for chem1 in neat Product A and of 12.8% for a 1:1000 ‘in use’ spray dilution.

The comparative *in vitro* study on human and rat skin demonstrated that, as with most substances, rat skin was more permeable than human skin and dermal absorption of the dilution was higher than that of the concentrate. The ratios of rat: human skin were 3.5:1 for chem1 in neat Product A and 4.9:1 for the ‘in use’ spray dilution.

On this basis the dermal absorption value for use in risk assessment is 1.3% (4.5%/3.5) for chem1 in Product A and 2.6% (12.8%/4.9) for the ‘in use’ spray dilution. **Values of 1% (for the concentrate) and 3% (for the in-use dilution) are therefore recommended for use in risk assessment.**

ANNEX III

PREDICTIVE VALUE OF *IN VITRO* STUDIES ON HUMAN SKIN

Franz (1975) compared the penetration of 12 organic compounds through samples of human abdominal skin under finite dose conditions with previously published results for dermal absorption of these chemicals through the forearm skin of human volunteers. The *in vivo* absorption was first based on urinary excretion over five days. However, results obtained after intravenous application were subsequently used to calculate total absorption. The flux was taken as the relevant parameter to describe the process of absorption, and generally a good correlation between *in vitro* and *in vivo* results was found. The only exception was thiourea, for which the *in vivo* experiment had to be repeated using the abdomen, rather than the forearm, to be in agreement with the *in vitro* prediction. With regard to total absorption as a percentage of the administered dose, *in vitro* testing revealed a higher or nearly equal (in two cases only) absorption than the *in vivo* experiment for nine substances. This suggests the prediction would be sufficiently conservative. However, with salicylic acid, caffeine, and dinitrochlorbenzene, approximately two to five times higher dermal absorption was obtained in the human volunteer study. The ranking of the substances for total absorption, based on median values, also revealed remarkable differences between the two sets of studies, although substances of low absorption *in vivo* (lower than 10%) were all clearly confirmed as such *in vitro*.

In rats, Bronaugh et al. (1982) examined the dermal absorption of benzoic acid, acetylsalicylic acid, urea, and caffeine by summing up the urinary excretion and an estimate for the amount retained in the body that was calculated from the achieved blood concentrations. The absorption values in terms of percentages of the applied doses were in excellent agreement with *in vitro* data (rat, full thickness skin) for all substances except caffeine. For urea, acetylsalicylic and benzoic acid, the absorption rates were in the same magnitude as determined by Franz (1975) in humans or on human skin. It must be taken into account that, in both studies, skin analysis was not performed.

Van de Sandt et al. (2000) postulated that it is difficult if not impossible to directly compare *in vivo* data with *in vitro* data because of the very different experimental conditions. Therefore, they tried to perform studies under standardised conditions with respect to dose, vehicle and exposure duration to overcome this lack of comparability. Their experiments with the pesticide propoxur demonstrated that the outcome of the *in vitro* study on human skin using viable full-thickness membranes (1.7 or 9.7% dermal absorption after 4 or 24 hours, respectively, including the amount that was retained in skin) correlated well with the human *in vivo* situation (mean values of 0.5 and 3.7%, based on urinary excretion). The actual *in vivo* absorption might be higher because the amount in skin remains unknown. Nonetheless, the level of dermal absorption is likely to be slightly overestimated if only the *in vitro* study is available.

For the more lipophilic fungicide ortho-phenylphenol, the same group found a 33% penetration through human viable skin at 48 hours after commencement of a four-hour exposure period, whereas about 15% of the total dermal dose had been excreted in the urine of human volunteers within the same time interval. Thus, good predictivity and sufficient conservatism in the estimation of dermal absorption by an *in vitro* experiment on human skin was again demonstrated (Cnubben et al. 2002), even though there might be doubts about the integrity of skin *in vitro* when the study duration exceeds 24 hours. This could explain

overestimation of dermal absorption *in vitro*. Penetration through an epidermal membrane was much higher in the study (nearly 100%), and the authors suspected some deposition in the dermis as previously hypothesised for lipophilic substances.

Van Ravenzwaay and Leibold (2004) compared the dermal absorption of 14 pesticides (including eight fungicides, five herbicides and one insecticide, all developed and manufactured by a single company) at two or three exposure levels *in vivo* in rats and *in vitro* on rat and human skin. Skin-bound residues were included. It could be shown that, for all substances, the *in vitro* results on rat skin usually overestimated the dermal absorption as measured *in vivo*. Only very few data were in the same magnitude; whereas for the majority of compounds and dilutions, the *in vitro* absorption was generally two to nearly 80 times higher. Furthermore, rat skin proved to be more permeable than human skin in all cases, although the individual ratios, as well as those for the comparison of *in vivo* and *in vitro* in rats, differed very much among the various substances.

Williams (2006) reviewed a number of studies for comparability of *in vivo* and *in vitro* dermal absorption of chemicals from different regulatory fields and found the *in vitro* data in general sufficiently predictive. However, some reservations were expressed about the use of such studies in estimating the dermal absorption rate of very lipophilic substances due to the strong reservoir effects to be expected *in vitro* that perhaps may not be similar enough to the *in vivo* situation.

A more comprehensive overview of the literature is given in EHC 235 (WHO 2006), which drew the conclusion that there is a generally good correlation between *in vivo* and *in vitro* dermal absorption data, and that properly conducted *in vitro* measurements can be used to predict *in vivo* absorption. The best results in terms of a similar absorption rate were achieved when viable full-thickness human skin membranes were used for the *in vitro* experiments and when the absorbed percentages of the applied total dose were compared instead of flux.