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GUIDANCE NOTES ON DERMAL ABSORPTION STUDIES

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Second Edition

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Environment Directorate
ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT
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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 aim to establish appropriate dermal absorption values for human health risk assessment of pesticides and biocides. The first version of this document was revised in 2018-2022 based on the experience gained in the assessment of dermal absorption studies for pesticides' risk assessments under regulatory frameworks in Europe, USA and Canada. Some aspects of this guidance might 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, as most references and examples of data in these Guidance Notes are related to pesticide chemicals, the document is primarily applicable to pesticides' and biocides' risk assessments.

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 estimate a human dermal absorption value. It is beyond the scope of these Guidance Notes to provide a harmonised regulatory or scientific decision framework.

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 and regulatory authorities (e.g. EFSA 2012; EFSA 2017 and USEPA 1998 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. However, these Guidance Notes provide recommendations for performing and interpreting dermal absorption studies with pesticides and biocides in order to reduce variability among studies and to improve and harmonise data interpretation. Also, this document supports the concept of reduction, refinement, and replacement of animal tests, as the use of the in vitro approach should be preferred unless otherwise required.

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 pesticides 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 or the data interpretation for the assessment of public health and occupational safety of chemicals, these Guidance Notes do not attempt to reconcile these differences of approach.

The project to develop the first edition of these Guidance Notes 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 values. Absorption of a chemical through human skin, following dermal exposure, determines the actual dose absorbed by the dermal route. In cases in which dermal absorption data for a chemical is unavailable, a default dermal absorption value is used for estimation of the dermally absorbed dose. The use of different default dermal absorption values by different regulatory authorities may affect the risk assessment outcome.

Two meetings were held (2005 and 2006) to frame the scope of the Guidance Notes, acknowledging significant differences between the approaches used in North America and in the European Union for dermal risk assessment.

The Guidance Notes were circulated for comments to the Working Group of National Co-ordinators of the Test Guidelines Programme (WNT) and WGP in May 2008 and October 2010. The first edition of the Guidance Notes was approved by the WNT in April 2011.

The first version of the Guidance Notes for the Estimation of Dermal Absorption Values, published in 2011, was revised in the period from 2018 to 2022 by the OECD Expert Group on Dermal Absorption, led by the European Food Safety Authority (EFSA) and the German Federal Institute for Risk Assessment (BfR). Several sections of the Guidance Notes were updated based on the assessment of new evidence from dermal absorption studies conducted with pesticides (Aggarwal, 2014; Aggarwal, 2015; EFSA, 2015; EFSA, 2017; Allen, 2021). Moreover, the references in the document have been revised, even if not systematically.

The second edition of the Guidance Notes went through an iterative process of review and commenting by an Expert Group on Dermal Absorption and the WNT in 2018, 2019 and 2022. The second edition was approved at the 34th meeting of the Working Party of the National Coordinators of the Test Guidelines Program in April 2022. This document is published under the responsibility of the Chemicals and Biotechnology Committee.

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 extent of dermal absorption, such as exposure time, product formulation, dose, dermal loading, 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 many other classes of chemical.

2. The assessment of dermal absorption studies was identified as a technical issue that could be a challenge to international harmonisation 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 conducted with pesticides or biocides and of guidance on estimating dermal absorption values in the absence of such studies. The Guidance Notes were prepared with the aim to establish dermal absorption values for occupational health and public health risk assessment of pesticides and biocides. Even if other regulatory frameworks than pesticides developed specific guidance documents (e.g. for cosmetics: SCCS, 2018), this guidance may also be relevant for other groups of chemicals or uses in other types of products where dermal absorption may be evaluated, thus enabling a consistent approach. The purpose of this document is to provide:

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

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 from guideline studies.

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 dermal absorption 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 for estimating a dermal absorption value for human health risk assessment. 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). In addition to providing guidance on evaluating this type of study, Section 7 also includes recommendations for performing dermal absorption studies with pesticides or biocides. Some considerations around in vitro studies include the following aspects:

- The rat model is considered to over-predict dermal absorption in humans, however rat in vitro or existing rat in vivo data can be used.
- Outcomes from analysis of human in vitro data vs. dermal absorption values derived from a "triple pack" approach indicate human in vitro data can be used as stand-alone data to predict dermal absorption for dermal exposures to pesticide products (EFSA, 2011; Allen, 2021).

7. In vivo studies (Section 5) should be conducted using OECD TG 427 (OECD 2004a) or similar protocols (e.g., OPPTS 870.7600). Sections 7 and 11.6 provides guidance on evaluating this and other study types, including ADME and human in vivo studies.

- As rat skin is more permeable than human skin, an appropriately conducted in vitro or in vivo study in rat is unlikely to underestimate dermal absorption in humans. No new in vivo studies can be conducted in Europe.

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). Application of the triple pack data to determine dermal absorption values can vary between regulatory authorities. As discussed above, several countries and regions have begun accepting in vitro data alone, so caution should be used when conducting or requesting new in vivo data for use in a triple pack.

- 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 (refer to sections 6.2 and 11.3).

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 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 in the study is very closely related to the commercial formulation in terms of solvent, surfactant content, potential for skin irritancy, skin sensitisation and concentration of ingredients. Section 7.2 also provides considerations for transferring data to untested products (read-across).
- Co-formulants (either additional actives or adjuvants) in the test preparation may have a significant impact on the dermal absorption of the primary active, and the outcome of a study in terms of flux or percentage absorption of the applied dose can be different when another formulation is used. Section 7.2 contains a table of solvents and co-formulants known to affect dermal absorption.
- Because of physicochemical considerations, and taking the above-mentioned aspects into account, dermal absorption data on another (reference) formulation can be used if the formulation for which dermal absorption needs to be determined is closely related (see Section 7.2.2.). Formulations may not be closely related when moving from one formulation type to another (e.g. suspension concentrate to emulsifiable concentrate).

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

- Common exposure locations include the back (in vivo studies) or breast/abdomen/back or upper leg (in vitro studies), but other anatomical locations may 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 a suitable surrogate, as studies with pesticides have indicated that dermal absorption from these areas 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 biocides, 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. Different regulatory agencies/organizations utilise different approaches/default values according to their policy and practices.

- In North American countries, 100% dermal absorption is used.
- In Europe, a significant impact on dermal absorption of concentration status (‘concentrate’ or ‘dilution’) and formulation category (‘organic solvent-based’, ‘water-based/dispersed’, ‘solid’, or ‘other’) is observed for pesticides (not corrosive), therefore default values have been derived accordingly (EFSA, 2017).
- To cover a ‘worst case’ scenario for corrosive formulations, 100% dermal absorption is assumed in both Europe and North American countries.

13. Other approaches are available to estimate dermal absorption in the absence of data. These other approaches are outlined in Section 10 and have different acceptability under the different jurisdictions. They may be used when no chemical specific dermal absorption data is available, as an alternative to a default value. 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' (skin penetration prediction of a test substance on the basis of experimental data obtained with a 'similar' compound, preferably from the same chemical group or class) is applicable only to chemicals that have been demonstrated to be similar in their chemical structure and physicochemical properties. General guidance on read-across and the formation of chemical categories are published (OECD 2014; ECHA, 2017).
- Modelling/QSAR can be useful in certain circumstances, though models built for specific chemical classes are currently of limited applicability, and in this case, the 'training set' must contain a reasonable number of closely related compounds. Models are difficult to use to predict the absorption of formulated products or preparations containing multiple chemicals, and formulation and skin conditions may not be taken into account. Even then, the formulated product may contain several adjuvants and the interaction between components makes prediction in silico difficult.
- 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, vehicle and formulants used may not be relevant to field exposures. Further, ADME data usually do not provide information on formulations.
- The use of dermal and oral data from toxicity studies has different acceptability under the different jurisdictions. Data from repeat dose oral studies (with oral absorption data) and repeat dose dermal studies may only be used where there is close similarity between the two studies in terms of design and systemic effects seen (Section 11.5). Data from oral and dermal acute toxicity studies should not be used.
- The use of other study types, such as human in vivo dermal absorption data has different acceptability under the different jurisdictions (Section 11.6).

PART 1: INTERPRETATION OF ROUTINE DERMAL ABSORPTION STUDIES CONDUCTED ACCORDING TO TG 427/428

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. For pesticides, humans may be exposed in non-occupational settings when applying pesticide products or after entering an area that has been treated with a pesticide product (i.e., post-application). In occupational settings, workers may be exposed when mixing/loading/applying pesticide products or when entering treated areas.

15. 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 subchronic dermal toxicity study, and a subchronic inhalation toxicity study if exposure via the dermal and inhalation routes are of concern for the risk assessment, respectively. Further, dermal toxicity studies are not suitable for evaluating dermal absorption to pesticides (e.g. due to high dermal doses). Loading conditions have an impact on absorption; % absorption diminishes with increasing load (Frasch et al., 2014)

16. As a result of these issues, oral studies are used as the basis for estimating the risk of exposure via dermal and inhalation routes. Furthermore, certain jurisdictions may use oral studies rather than route specific studies as the route specific study may not address specific toxicities of concern (e.g., neurotoxicity, increased susceptibility, or when a target organ was not assessed). 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).

17. Dermal absorption studies are conducted using in vivo methods (OECD TG 427, US EPA 870.7600) and in vitro methods (OECD TG 428). Ethical considerations have historically discouraged the use of human, and, increasingly, non-human in vivo dermal absorption studies to evaluate dermal penetration. Therefore, though in vivo studies are available, in vitro studies using human or animal skin are conducted more frequently than in the past. With respect to pesticides and biocides, the results of the in vitro dermal absorption studies can be used alone or in conjunction with other existing data available for pesticides risk assessment purposes (Section 4).

18. 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

19. For the determination of dermal absorption values of pesticides for regulatory purposes, in vitro studies can be used in one of the following two ways:

- Use in vitro human skin dermal absorption data as stand-alone to predict the expected dermal absorption by humans under field conditions without further conversion or correction, or;
- Some regulatory authorities use in vitro studies 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 or identically prepared, and the applied concentrations were at least similar (i.e. the 'Triple Pack' approach, see Section 6).

20. The OECD Test Guideline 428 (OECD, 2004c) provides a protocol for using excised human or animal skin for the purpose of assessing the dermal absorption of chemicals and formulated products and/or dilutions. However, this Test Guideline and its accompanying Guidance Document No. 28 (OECD, 2004a) were written for general use, and generally do not include sector- or chemical property-specific protocol recommendations. Since then, sectorial guidance documents and other documents have been developed on the conduct and interpretation of dermal absorption studies, including additional recommendations to the TG428 protocol aiming to improve study protocol standardisation, to reduce experimental variation and aid consistent data interpretation from dermal absorption studies with pesticides (e.g. EFSA 2012; EFSA, 2017; Sullivan, 2017).

21. During the European Union evaluation process for pesticides under Regulation (EC) No 1107/2009, the dermal absorption values of many pesticides are estimated using data from in vitro studies on human skin (e.g. EC 2003a; EC 2003b; EFSA2011; EFSA, 2012; EFSA, 2017). Based on an analysis of human in vitro data versus triple pack data (Dewhurst, 2010; EFSA, 2011), it was concluded that human in vitro data can be used as stand-alone data to predict dermal absorption of pesticide products as both concentrates and dilutions

22. In North America, recent literature provides recommendations to facilitate comparative data analyses, aiming at achieving a wider global acceptance of in vitro dermal absorption studies alone for pesticide risk assessment (Sullivan, 2017). A retrospective analysis of triple pack data has showed that the in vitro method provides similar or higher estimate of dermal absorption in vivo, supporting the use of human in vitro data alone for derivation of dermal absorption values for pesticides risk assessment (Allen, 2021).

23. 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.

4.2 Species selection

24. For the risk assessment of pesticides and biocides, in vitro dermal absorption studies on human skin or rat skin (or both) are usually available. If reliable studies on human skin are available, these should be given preference over studies performed on skin from other species when used in stand-alone derivation. In general, rat skin is considered to be more permeable than human skin (Handbook of Pesticide Toxicology, 2001).

25. In general, human skin is less permeable than that of laboratory animals with pig or non-human primate skin being considered the best predictive model for human percutaneous absorption (Monteiro-Riviere, 2008; WHO, 2006; Holmgaard and Nielsen, 2009). Although pig and non-human primate skin has been shown to be a good surrogate for human skin, it is noted that there are issues related to additional testing and validation to conclude on 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 using skin 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.

26. Previously, a deterrent to the wide acceptance of in vitro data was largely related to their high variability, demonstrated in an inter-laboratory comparison conducted in 2004 (van de Sandt et al. 2004). In the case of this study and other similar studies, the major cause for this variability has been identified as the high degree of flexibility in the test protocol for both protocol elements and data reporting. However, increasing standardisation of experimental conditions outlined in OECD TG 428 (OECD 2004b) are helping to reduce this variability and allow a consistent study conduct and data interpretation. These experimental and reporting conditions are outlined in sectorial guidance documents (EFSA, 2012; EFSA, 2017) and the literature (Sullivan, 2017).

27. Human skin for in vitro studies is either taken from autopsies (cadaver skin) or obtained during aesthetic surgery. Permeability of human skin can vary, 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 (different anatomical sites in humans display a hierarchy of absorption: scrotum > forehead > torso and arms > palms and soles of feet (e.g. Weltfriend et al, 1996; Maibach et al., 1971). Recommendations on the skin samples to be used for rat and human dermal absorption studies are provided under paragraph 34. Further, the stratum corneum barrier function of individuals is expected to be different in the population due to a number of factors (sun exposure, diet, age, genetics etc.). This might be in contrast to in bred laboratory animals. Thus, an adequate number of replicates and donors should be used (refer to paragraph 30).

28. 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 are typically 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

29. For recently conducted studies, it can be expected, and it is generally required, that guideline OECD TG 428 (OECD 2004) has been followed. These Guidance Notes acknowledge that not all available dermal absorption studies 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.

30. 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 stability and homogeneity of the dosing solutions, the description of the diffusion cells used, the actual area of skin dosed, the number of replicates/samples and donors (for each dose level to be tested: 8 replicates from at least 4 different donors (interpreted in most countries to mean at least 2 replicates from each donor), the duration of study/sampling period (preferably not more than 24 hours), the use of filter traps for volatile test material (e.g. porous carbon filters) as part of the mass balance recovery, and the determination of the amount retained in skin after washing including the washing procedure (should mimic normal practice) and, the tape stripping procedure (including the quantification in the tape strips). Additionally, validation of analytical studies might be crucial, especially for non-radiolabelled material.

31. If the amount of chemical remaining in the treated skin in vitro has not been analysed, the study will usually be considered as unacceptable because the amount of test substance remaining in the skin at the end of the study is considered as absorbed.

32. In vitro methods are designed to measure the penetration of chemicals into the skin and their subsequent permeation through 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). Then, in principle, the mechanism of skin penetration may be regarded as the same as in vivo, noting that some in vivo ADME processes are not covered in vitro.

33. 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, but the in vivo situation is not fully represented.

34. The thickness of the skin sample used is also a crucial point, such as full-thickness versus split-thickness, or all three layers (dermis, epidermis, stratum corneum) versus samples where either dermis or stratum corneum has been removed before applying the test item. In vitro studies with human and rat skin should preferably use split thickness (200-400µm) (dermatomed) skin (refer to OECD TG 428). Human skin should be from the abdomen, back, breast or upper leg and rat skin should be from the abdomen, upper flank or back. This is to improve consistency and comparability particularly as split-thickness membranes tend to have significantly lower levels of residual material than full-thickness preparations (OECD, 2004c; WHO, 2006; Wilkinson et al., 2006; EFSA, 2011). The use of epidermal membranes may, in some cases, overestimate in vivo skin absorption because of insufficient barrier function. The use of cultured and reconstructed human skin models (e.g. constructed from keratinocytes) is not recommended for the determination of dermal penetration as these models have not been validated for dermal absorption studies and there are reports that their barrier function is not comparable with that of skin of 'natural origin' (SCCS, 2010; WHO, 2006).

35. The integrity of human or rat skin used in vitro should be determined prior to application of the test substance and should be documented. Various methods can be used (e.g. trans-epidermal resistance, trans-epidermal water loss or reference substance penetration) (OECD, 2004c). Any membrane with unacceptable integrity should be replaced prior to application. Post-dosing evaluation of integrity instead of pre-dosing evaluation, and subsequent exclusion of results obtained with skin having insufficient integrity is not recommended because a sufficient number of reliable replicates should be ensured (see above,

paragraph 30). Post-dosing evaluation done in addition to pre-dosing might provide an option for the identification of outliers.

36. Both static (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 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.

37. 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 cleaning agent relevant to real-world practice 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 (i.e., 6-10 hours of exposure with additional sampling after washing up to 24 hours after initiation of exposure). At 24 hours, the skin surface should be washed again in order to fully characterise the un-absorbed or potentially absorbed dose as part of the mass balance procedure.

38. 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 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.

39. 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 (reservoir potential of the stratum corneum in addition to other skin layers). 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).

40. 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. The skin may act as a reservoir if the test substance accumulates in the skin instead of passing directly through to the bloodstream (Vickers, 1972). Depending on the physicochemical properties of the permeant and its interaction with skin components, e.g. proteins, the kinetic behaviour including timeframe for bioavailability, and elimination from systemic circulation, it may form a reservoir in the non-viable layer of the epidermis (stratum corneum), in the viable layers of the epidermis, or in the dermis. From the reservoir, the permeant may continue to diffuse further into the skin after exposure has terminated, in the end becoming systemically available via the lymphatic or sanguine capillary network in the dermis (WHO, 2006). In vitro methods do not provide information. on elimination from systemic circulation, or metabolism. Partitioning can be described in greater detail if the different skin layers have been analysed separately. It is generally agreed that residues in the non-viable stratum corneum layer are more likely to be sloughed from the skin over time (desquamation) and not become bioavailable. Residues in the viable layers of the epidermis and dermis are more likely to become bioavailable over time and are typically considered to be absorbed. In many studies, tape stripping is used to determine the percentage of the applied dose 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.

41. To increase confidence in in vitro results, some countries have suggested the presentation of data for reference compounds such as testosterone, caffeine, or benzoic acid 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 compounds.

4.4 Receptor fluid

42. 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)). It must be demonstrated that solubility of the active substance in the receptor fluid is sufficient. This can be expected if the solubility is at least 10 times higher than the expected (maximum) concentration of the test compound in the receptor fluid at the end of an in vitro study. 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.

43. 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).

44. 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.

45. 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.

46. Yang et al. (1986) 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.

47. Bronaugh and Stewart (1986) reported 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 trichlocarban 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%).

48. 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.

49. 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 (ANIMAL) DATA

50. The main advantage of in vivo animal data from guideline studies is that they are generated from a physiologically and metabolically intact system. 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. As rat skin is considered more permeable than human skin, this approach is unlikely to underestimate dermal absorption in humans. The approach could therefore be considered to be conservative. For further information and references see the WHO/IPCS Environmental Health Criteria 235: Dermal Absorption (WHO 2006). It is noted that no new in vivo studies on animals can be conducted in Europe and existing in vivo data only can be used. In keeping with the principle of the 3Rs, de novo in vivo studies should be generally avoided as acceptance of in vitro methods grows.

51. In some jurisdictions (e.g. North America countries), data from other species than rat (e.g. pig or monkey) 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.

52. There are several types of in vivo animal (rat) data that might be used for estimating the dermal absorption value. This section describes guideline dermal absorption studies using the rat. Other types of in vivo studies not covered in Test Guideline 427 (OECD, 2004) are discussed in Section 11.6.

53. In vivo studies for dermal absorption produce the most comprehensive 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 chemical in an appropriate test preparation and in typical formulations, spanning the realistic range of potential human exposure.

54. 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.

55. The dermal absorption value (including or excluding the dose in the application site as appropriate) from the final time point (the value from the group sacrificed with the longest post-wash observation period) is generally the most appropriate regulatory value for a study conducted according to OECD TG 427 or similar protocols (such as OPPTS 870.600). 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).

56. 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.

57. 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.

5.1 High Amount of Applied Dose in Dressings

58. For in vivo dermal absorption studies, the dosed site is typically covered with a semi- or non-occlusive material (e.g. bandages, gauze) to prevent the animal from gaining access to the site and ingesting the test material. This covering is typically removed at skin wash and a fresh set of coverings are applied if the study continues after the skin wash. Although coverings are installed so that they are not in contact with the dose site, test material is often detected on the coverings. This may occur from natural desquamation of the dosed skin, flaking of dried test material from the dosed site, or the covering material contacting the dosed site.

59. Low amounts of test material found in the coverings collected at the skin wash is allowable and does not need adjustment (e.g. less than 20% of the applied dose). However, higher amounts may impact the acceptability of the study as the dose on the coverings may not have been in contact with the skin for the length of the exposure period. Since dermal absorption is impacted by dose and exposure time, loss of large amounts of test material from the dose site before the skin wash can result in an underestimate of dermal absorption. Therefore, if more than half of the applied dose is found on the coverings collected at the skin wash, it may be reasonable to reject the study. For studies where less than half of the applied dose is found on the coverings, rather than reject the study, consideration could be given to adjusting the applied dose by the amount on the coverings which may not have been available for absorption.

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

6.1 Introduction: the 'Triple Pack' approach

60. 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 to get an estimate of human dermal penetration. The combined use of data from the three studies and two testing systems permits refinement of dermal penetration results by accounting for any difference between in vitro and in vivo absorption rates in that species as well as for species difference seen between the animal and human skin

61. 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:

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

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 (Dewhurst, 2010; EFSA, 2011; Allen, 2021). 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. The Triple Pack calculation is based on the original underlying assumption that a ratio of 1 or higher would be obtained when comparing in vitro and in vivo rat absorption conducted with high quality. It was suggested that the factor between the dermal absorption in vitro and in vivo will be the same for humans when the same technique is used. However, that ratio was not attained in all cases (Allen, 2021).

62. 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%)

63. Some regulatory authorities and organisations may apply the 'Triple Pack' approach as described above. It might be possible to use existing in vivo data in rats (or other experimental animals) corrected for the ratio of absorption between rats and humans in vitro in a triple pack approach.

64. The 'Triple Pack' approach should be used to estimate a dermal absorption value only when the three studies are conducted during an appropriate similar time period and importantly 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/ vehicle (for example, formulations such as emulsifiable concentrates or granules or in-use spray dilutions). To ensure scientific validity, this also means skin type (i.e. split-thickness), state of occlusion, sampling period, receptor fluid composition, skin wash technique, and analytical techniques for in vitro studies should be considered.

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

65. When acceptable (e.g. guideline-compliant and GLP) 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 , and, the results meet the quality criteria, in particular with respect to variability, number of acceptable replicates/animals and recovery (e.g. as described in EFSA (2017)), then the 'Triple Pack' approach can be used to determine the human dermal absorption values for risk assessment. In case the above-mentioned conditions are not fulfilled, it could be justified to reject the approach.

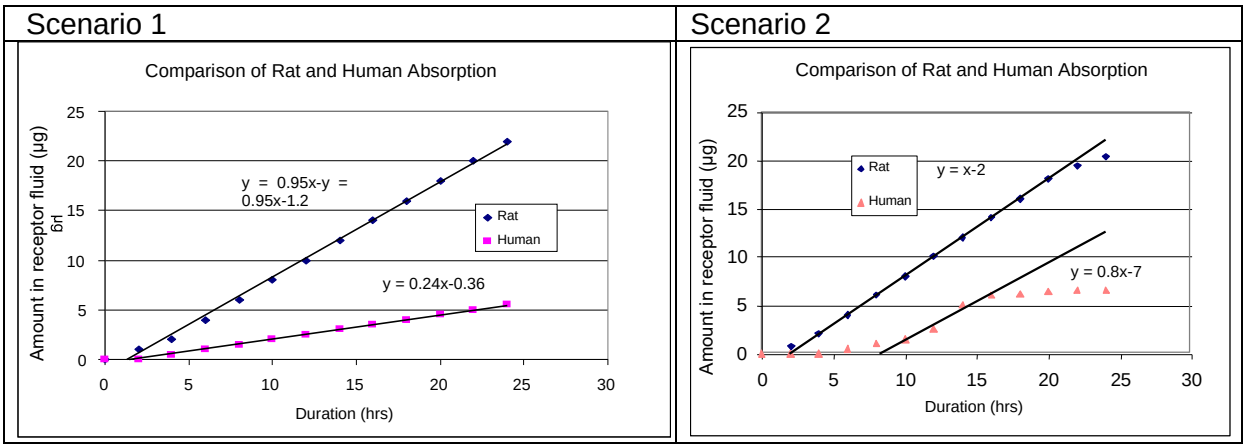
66. The question of whether to include skin-bound residues is addressed in Section 7.1. For in vitro studies, the OECD guideline (OECD 428) defines the 'absorbable dose' as 'that present on or in the skin following washing'. A similar approach is recommended for the in vivo studies.

67. 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. In general, infinite dose conditions are required to generate a steady state flux provided that the dose on skin never depletes. The flux describes the penetration of the substance per area unit (square centimetres, cm2) 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: Using flux values means that a penetration rate per time is used but ignores the subsequent penetration of the substance from a possible skin reservoir. In general, the flux is not recommended for use in the risk assessment of pesticides, and the percentage absorption is preferred (see Section 11.3). However, if flux is used then the following should be considered:

- 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.
- 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 in Figure 1 can demonstrate the difficulty of using flux:

Figure 1: Comparison of Rat and Human Absorption under Scenario 1 and Scenario 2



The calculation of the ratio of absorption between the species can be summarised as in Table 1:

Table 1: Calculation of absorption ratio under scenario 1 and 2

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

68. 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

69. 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 (after exposure and/or at the end of the experiment).

70. The current approach taken by nearly all regulatory agencies is to determine the dermal absorption value by adding the absorbed dose and dose 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 that is retained in the stratum corneum and can be removed by tape stripping, should be included in the dermal absorption value.

71. It is widely accepted that, if absorption can be demonstrated as essentially complete (see 7.1.3) then the chemical remaining in the stratum corneum may be considered as unavailable for absorption.

72. 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*

73. Tape stripping is a procedure performed at the end of a dermal absorption study that involves the sequential application of adhesive tape to the area of skin that was exposed to a chemical (Trebilcock et al., 1994). A standardised protocol for a tape stripping procedure was not included in OECD TG 428. If the tape strips are analysed individually, a profile of the chemical across the stratum corneum can be determined and may give some indications on the conduct of the study. However, the development of such a profile includes some assumptions and uncertainties including the assumption that each tape strip represents a single layer of stratum corneum, which is not necessarily the case due to furrows in the stratum corneum. Further, difficulties for tape stripping techniques may result in inconsistent fractions removed with each tape strip. Though not required, devices may be used to standardize the tape stripping process (Sullivan et al 2017). 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.

74. 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.

75. In the European Union and some other countries (e.g. United States), it is the practice for pesticides 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 EFSA guidance (EFSA, 2017) states that "[...] glued (e.g. cyanoacrylate superglue) tape strips should not be used" (or else the complete tape strip fraction must be considered absorbed).

76. 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.

77. Dermal absorption is 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 that absorption is complete and this test substance will remain in the stratum corneum (see 7.1.3) and exfoliation can be assumed.

78. If separate analysis of the individual tape strips has not been performed and all tape strips are pooled before measurement, 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 (EFSA, 2017; EFSA, 2011). 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 and in vitro

79. Following an in vivo animal study or an in vitro study, the 'absorbable dose' refers to 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. In some jurisdictions (e.g. Europe) if absorption is determined to be essentially complete by the end of the study, all material in the stratum corneum (tape stripped material) can be excluded from calculation of the absorbable dose. This could be applied to both in vitro and in vivo studies:
   a. An in vivo dermal absorption study is generally 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.
   b. When there is evidence that absorption is nearing completion (e.g. marked decline in the amount over the last three sampling times), where less than 75% is absorbed by the end of the study, material from all tape strips can be excluded from the absorbed material if the evidence indicates that it is not bioavailable (e.g. study run for 168h and the majority of the material is in the tape strips from the upper layers of the stratum corneum). However, this should be critically assessed on a case-by-case basis.
   c. For in vitro studies, permeation is considered essentially complete when more than 75%* of the amount that has permeated into the receptor fluid at the end of sampling (usually at 24 h) has reached the receptor fluid at the half time of the sampling period (usually at 12h). For an in vitro study with a sampling period of 24h, the mean relative permeation into the receptor fluid occurring within half of the sampling period (t0.5) should be calculated from the individual replicate data on amounts recovered in receptor fluid (RF) at 12h (RF12) versus 24h (RF24). If the t0.5 value is close to 75%, a confidence interval should be estimated to demonstrate credibility of the conclusion that permeation is essentially complete (EFSA, 2017 section 5.1).

* 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 had not been validated with use of real-world data at the time of this publication.

Figure 2: Examples of representative absorption (as a percentage of the total) vs time profiles

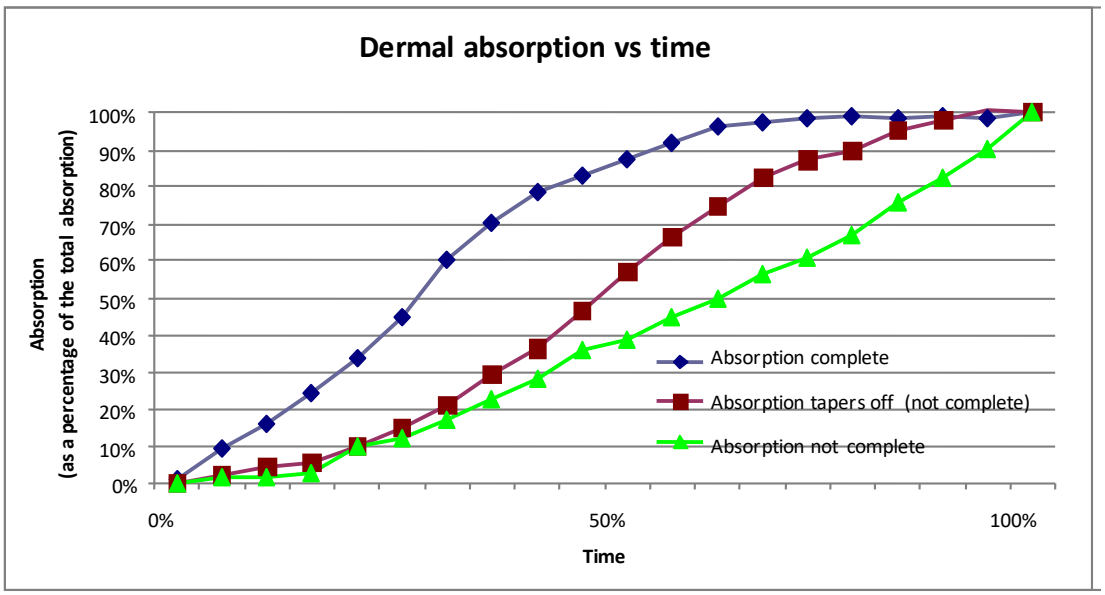
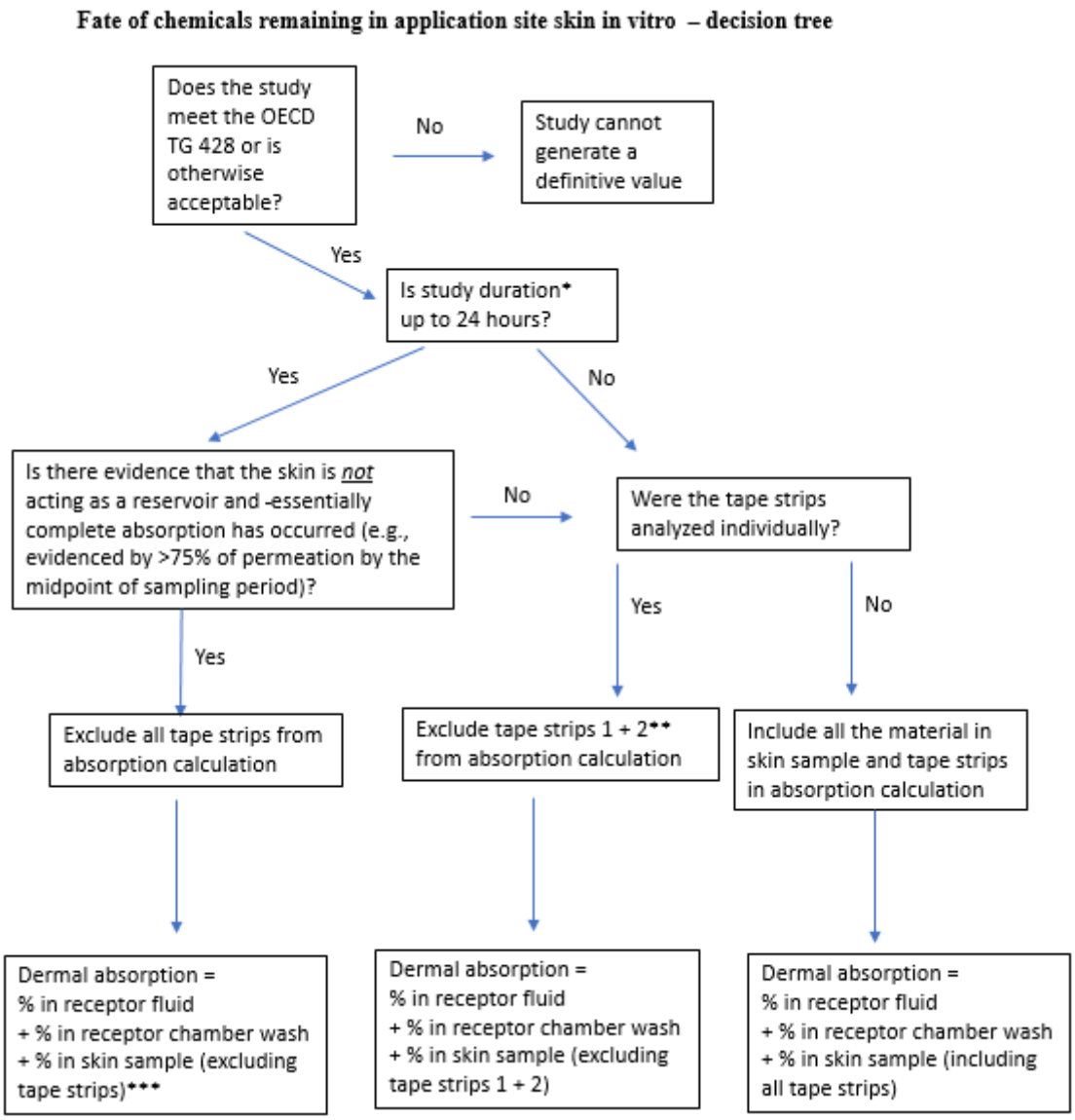


Figure 3: Fate of chemicals remaining in application site skin in vitro – decision tree

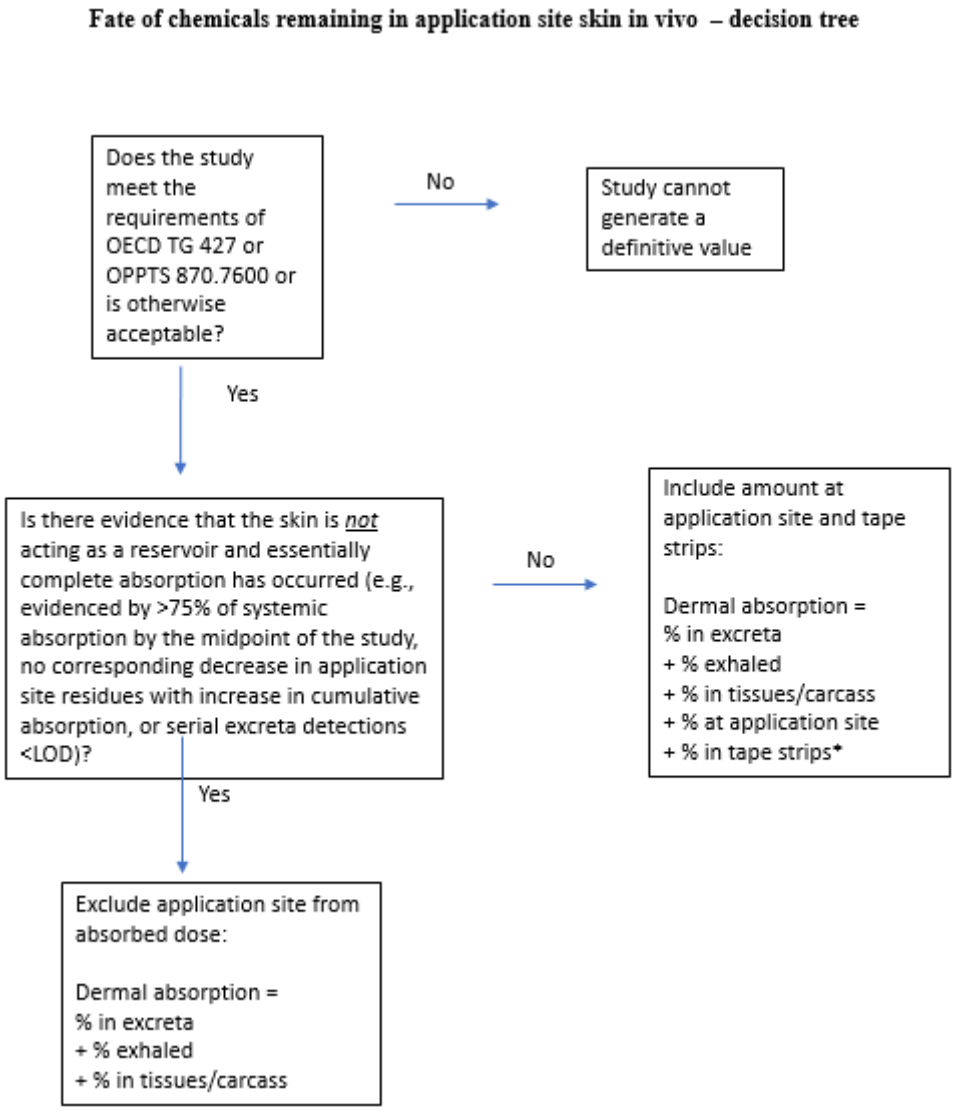


* Duration refers to the study and not to the exposure of the skin

** In Europe and some other countries (e.g. United States), the first 2 tape strips are excluded as it is assumed that they represent material that will not become bioavailable due to desquamation

*** In some jurisdictions (e.g., United States) the skin sample may be excluded altogether on a case-by-case basis

Figure 4: Fate of chemicals remaining in application site skin in vivo – decision tree



* In Europe and some other countries (e.g. United States), the first 2 tape strips are excluded as it is assumed that they represent material that will not become bioavailable due to desquamation

7.2 Effect of formulation

7.2.1 Test preparations

80. Test preparations are either commercially available formulations (for example, plant protection products with their respective approved field dilutions or biocidal products with in-use 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 (including additional actives or adjuvants) 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 2 for a list of solvents and co-formulants known to affect dermal absorption.

81. 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 14C-radiolabelled, but non-radioactive material can be used if appropriate and if validated analytical methods have been established (FDA, 2018).

7.2.2 Influence of formulation

82. 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 (Hilton et al, 1994). 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.

83. Small differences in the test preparation can greatly influence the in vitro penetration profile. Further, partitioning can be enhanced or evaporation of the vehicle may impede penetration, e.g. 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.

84. Regulatory authorities have recognised the influence of the product formulation on dermal absorption. The EPA-870.7600 test methods for dermal penetration (USEPA 1996) recommend that the test formulation used should replicate that under which field exposure occurs. Likewise, OECD Test Guideline 427 and Test Guideline 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.

85. 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.

86. Dermal absorption data on another formulation can be used if the formulation for which dermal absorption needs to be determined is closely related. Formulations can be considered as similar, if the active ingredient and content of relevant components in the formulation to be assessed (e.g. co-formulants, other active substance, synergist, safener, wetting agent, surfactant, solvent, emulsifier, preservative, stabiliser, detergent, adhesive, antifreezing substance) are within acceptable variation ranges of those in the reference formulation. E.g., the impact on dermal absorption of a co-formulant at 10 % (tested formulation) may be considered the same at 12%.

87. Formulations can be considered as similar, if the active ingredient and content of relevant components in the formulation to be assessed (e.g. co-formulants, other active substance, synergist, safener, wetting agent, surfactant, solvent, emulsifier, preservative, stabiliser, detergent, adhesive, antifreezing substance) are similar. In Europe, to determine if the co-formulants are similar, Regulation (EC) No 1272 /2008 and the guidance on significant and non-significant changes (Sanco/12638/2011, 20 November 2012 rev.2) provide bridging principles for hazard assessment of mixtures (see table 3 in EFSA Guidance (EFSA, 2017)). It should be noted that these permitted variations were not determined from dermal absorption data and the impact of variations outside these values on dermal absorption has not been determined. Refer to the discussion below for co-formulants that are outside the permitted variation.

In individual cases, greater variations might be acceptable, for example in the case of the replacement of a co-formulant by water or in the case of an increase of an inert compound.

88. Co-formulants of both formulations should be chemically and physico-chemically closely related (e.g. toluene and xylene; octanol and nonanol; linear alkyl sulphonate is not acceptably replaced by an aromatic sulphonate derivative). They should also be similar with respect to interaction with the active substance (e.g. solubility or enhancement of toxicological properties). However, the evaluation of similarity should be determined on a case by case basis, and within a given regulatory framework.

89. Table 2 below 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.

Table 2. 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), Williams et al. (2004)
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), Abd et al. (2019).
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)
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 (sodium lauryl sulfate) 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)
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)

90. In addition to the specific chemicals present in a formulation, pH is also an important consideration to determine if the formulations are closely related 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 the dermal absorption in this way. pH is not available for every formulation, e.g. for solid or non-aqueous liquid products. However, data may be available for related liquid solutions, e.g. in the EU (Reg. (EU) No 284/2013), data on pH have to be provided in such cases as aqueous dilutions of a 1% dilution.

91. Irritation and sensitising properties might have relevant impact on dermal absorption. Sensitizing effects are allergic responses in susceptible individuals, in which, after a first initialising (sensitising) dermal exposure, subsequent dermal exposures may cause allergic contact dermatitis or atopic dermatitis, characterised by symptoms comparable to erythema, oedema, and vesiculation, but immune-based reactions. Thus, besides irritants, the presence of sensitizers may cause significant skin effects resulting in increased skin permeability due to compromised skin barrier (e.g. WHO 2006). However, there is currently limited investigation of the impact of sensitization on dermal absorption. The formulations under evaluation should have the same or no sensitising potential based on classification and it should be of the same or lower skin irritancy based on scores in studies. These should include initial findings (as dermal absorption is often significant within the first 24 hours), not just the classification.

92. In Europe, to determine if the active substance concentrations are similar, the active substance concentration should be within permitted variations of that in the reference formulation (see table 4 in EFSA Guidance (EFSA, 2017)). This Table is based on the FAO and WHO specifications for pesticides concentrated products (FAO/WHO, 2016, chapter 4.3.2) and thus the reference formulation should be understood as e.g. concentrated dose/product, formulation representative of the undiluted test formulation. The permitted variations provided take into account manufacturing, sampling and analytical variations, in contrast to permitted variations for co-formulants presented above, which are based on physical-chemical considerations. As noted for the co-formulants table above, these general principles are used in some jurisdictions; however, they are not based on dermal absorption data and the impact of variations outside these values on dermal absorption has not been determined. If the active substance concentration in the tested formulation and the registered (untested) product are within the permitted (relative) variation then the results of the tested formulation are acceptable to use for that product. If the active substance concentration differs by more than the permitted variation, then additional guidance in the subsequent paragraphs (e.g., 86) should be followed, to determine if further data are required.

93. 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. Nevertheless, due to the fact that exceptions do exist, it should be demonstrated that the percentage dermal absorption is inversely related to the concentration of the active substance. For example, an estimate from a lower concentration may not result in a conservative estimate for skin-irritating or volatile substances (Buist et al. 2009), where it cannot be ruled out that the percent dermal absorption of skin-irritation substances increases with dermal load of substance (mg/cm2).

94. Pesticides are often marketed as concentrate and diluted for the application. Thus, the tested concentration(s) should cover the extremes of those recommended on the product label. If the lowest concentration tested is greater than the lowest concentration of the same formulation recommended on the label, different approaches are taken under the different jurisdictions. For example:

- In Europe, consideration should be given to increasing the dermal absorption pro rata to account for any limitation of absorption due to the amount of material applied to the test site. Pro rata correction assuming a proportional response is considered to be a conservative but appropriate

approach in the absence of data and is a concept of worst-case linear extrapolation which applies to dilutions (refer to EFSA Guidance 2017). In case the same formulation and its dilution(s) are tested, this approach can be used to derive dermal absorption values for other dilutions with concentrations of the same formulation lower than the highest dilution (lowest tested active substance concentration). However, if the dermal absorption from the concentrate, a dilution and the lowest tested concentration (second dilution) shows no indication of concentration related absorption, then there is no need to increase the value for the lower (untested) concentration of the same formulation recommended on the label.

- In North American countries, conservative values are often selected to be applied to a wide range of scenarios and products. The process for determining these values depends on the tested doses and formulations, their similarity to the reference formulation, and if dermal absorption is inversely proportional to dose.

7.2.3 Solid vs. liquid formulations

95. A wide range of different product formulations may be available for a given pesticide/biocide such as emulsifiable concentrates, granules (without solvent only), wettable powders, and water insoluble powders. Because of physicochemical considerations, it may be assumed that skin penetration of solid materials (such as granules) will be equal to or less than for liquid formulations (water-based or 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 a liquid-based test preparation may be considered as a 'worst case' for solid/granular formulations. However, this may not be the case if the solid formulation is added to water or other solvent and applied as a liquid. For liquids, small differences in the test preparation can greatly influence the penetration profile as reported above. Read across for different liquid formulations and even spray-dilutions independent of the formulation type would ignore the different levels of detergents, surfactant, emulsifiers etc. which is considered to impact dermal absorption even in dilutions. Referring to the dataset on in vitro dermal absorption studies on human skin which was analysed for default derivation of pesticides (EFSA 2017, refer to section 9; dataset available at https://zenodo.org/record/3378822#.XWfi4-gzaUk) it can be argued that solid formulations and respective spray dilutions in general seem to be more similar to water based formulations than to organic solvent based formulations. However, it should be noted that the solid based formulations in this dataset were primarily formulations that would be mixed with water or solvent and applied as a liquid.

7.3 Metabolism in the skin

96. 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).

97. 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.

98. 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. (2000a and b), Bronaugh (2004a and 2004b) and WHO (2006).

99. 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, literature has shown that metabolism could affect absorption. Hewitt et al.(2000a and b) confirmed the hydrolysis of ester compounds in viable skin layers. They demonstrated that the rate-limiting step of metabolism was the diffusion from stratum corneum into the layers beneath it. Up to now, no accepted methods are available to consider this aspect under the evaluation of dermal absorption for pesticides risk assessment. Metabolism 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.

100. 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.

101. 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 improve 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

102. OECD TG 427 and 428 (OECD 2004a and 2004b) aim for 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. OECD TG 427 and 428 provide methods for measuring skin absorption of all chemicals. Generally, as stipulated in OECD 428, laboratories should be able to demonstrate that they can perform in vitro dermal absorption studies to available standards and provide evidence for this with dermal absorption data on the named OECD reference chemicals. General aspects for the study conduct have to be checked, i.e. purity, homogeneity and stability of all the pesticide applications, the use of filter traps for volatile substances, correct skin storage and preparation, check of skin integrity, proper tape stripping procedure.

103. The mass balance recovery criteria outlined in OECD TG427/428 are followed by many jurisdictions, including North American countries, however, some jurisdictions may decide to utilize a more stringent criteria. For example, the EU considers a mean mass balance recovery for pesticides between 95–105% justified based on an analysis of a large dataset (available at https://zenodo.org/record/3378822#.Yeq51-rMKUk) of in vitro studies on dermal absorption of pesticides using human skin that demonstrated that a higher recovery is achievable in most cases (83% meet a recovery >95%) (EFSA 2017). These higher recoveries have been attributed to modern techniques and procedures that have developed to improve the in vitro method (Heylings et al. 2018) and, despite some

divergent views on their applicability (Kluxen, 2019; Heylings, 2020), are considered to reflect laboratory quality standards.

104. The criteria to justify mean mass balance recovery values outside the respective 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. In any case, the upper limit of recovery range is an indication of study quality and strong exceedance should be considered critically.
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.

105. If the results from some individual replicates (animals or in vitro test units) 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. Losses that are considered to be from non-absorbed material will have no impact on the results. If losses appear to be from absorbed material, the values could be corrected for the losses by considering the lost fraction as absorbed. A few options for correction are discussed below.

106. If losses appear to be from adsorption to the equipment, this must be demonstrated in a procedure where the equipment used in the study is washed and wash solution analysed. Depending on the piece of equipment, the amount in the wash would be considered absorbed (e.g. cage wash, receptor chamber) or not absorbed (e.g. donor chamber). If the equipment from the study is analysed, then the residues in the wash solution can be added to either the absorbed or non-absorbed amounts from the study.

107. 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%, or 5% acc. to EFSA (2017) 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 low recovery would be expected for all animals and correction for recovery could be performed by assuming that all the missing radioactivity could have been absorbed. If there are some replicates/animals with adequate recoveries, then the results for low and acceptable recovery animals/replicates exposed to the same dose can be compared to see if the losses are from absorbed or non-absorbed material. For example, if the amount in the absorbed matrices (e.g. receptor fluid, or carcass and excreta) are similar for the replicates/animals with low and acceptable recovery and the amount in unabsorbed matrices (e.g. skin wash) are lower for replicates/animals with low recovery, then it could reasonably assumed that the losses are from non-absorbed material.

108. If the mean recovery is below the recommended range for pesticides across test animals or replicates, and the fate of the unrecovered material is unclear, there are three potential approaches. Two approaches take into account the unrecovered material on a case-by-case basis and a third approach where low recovery replicates may be excluded:

- One approach would be to normalise the measured dermal absorption value. Values for all animals/replicates with a recovery below the respective acceptable recovery range should be normalised individually. Adjustment of those rats/cell where recovery was acceptable is not required. For example, if the measured dermal absorption was 10% of the applied dose, and the recovery 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 assumes that losses occurred in all matrices equally.
- A second 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 characterised as a worst-case assumption. This approach should be given the preference if the fate of the unrecovered material is unclear but likely to be from an absorbed matrix (appropriate worst case). In Europe, when mean recovery is below 95% and the calculated dermal absorption value is below 5% (EFSA, 2017), the missing material should be considered as absorbed and therefore added to the absorbed amount as worst case. If recovery is <95% but a robust explanation demonstrating the missing material would not have been or is very unlikely to have been absorbed, then the inclusion of the missing material might not be required.
- A third approach would be to exclude the replicates with low recoveries and only the replicates with high recovery should be used to derive the absorption. However, as exclusion reduces the overall number of replicates, a balance must be found between uncertainty resulting from low recoveries vs. uncertainty from a lower number of acceptable replicates (see section 7.5.3 on variability).

7.5 Use of mean or centiles; treatment of variability, outliers, non-detects and use of rounding

7.5.1 Introduction

109. 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 for the in vitro studies, such as differences between donors of human skin samples, body site, stratum corneum barrier, and for both in vitro and in vivo studies, slight damage to skin samples and application sites during preparation, dose application or skin decontamination. 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 within the results

110. In some jurisdictions, if variation between replicates for an in vivo study is not considered adequate (e.g. the standard deviation is equal to or greater than 25% of the mean), then a value other than the mean or possibly rejecting the study entirely may be considered. 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 (e.g. the standard deviation is equal to or greater than 25% of the mean) and group size is small (n < 4), the dermal absorption may 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, some jurisdictions may decide to add 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 could reduce the need to repeat studies. In in vitro studies using human skin, the level of acceptability is related to the number of skin donors and replicates used in the study. As noted in Section 4.3 (paragraph 30) a minimum of 4 different donors is required for each test group. If the test group uses fewer donors, then the high variability is less likely due to donor variability and more related to how the study was conducted. In this case, consideration should be given to using a value other than the mean, as discussed above for in vivo studies.

111. In Europe, the preferred approach to addressing uncertainty about mean absorption due to variability between replicates/animals is to add a multiple of the sample standard variation to the sample mean value. The multiplication factor required depends on the number of replicates (e.g. 1.6 for 4 replicates, 0.84 for 8 replicates, etc; see table 1 in the EFSA Guidance (EFSA (2017)). The result of this calculation approximates the upper limit of the 95 % confidence interval for the mean absorption and was validated using data from 420 human dermal absorption studies with pesticides (EFSA, 2017; dataset available at: https://zenodo.org/record/3378822#.XX0k2CgzbD5). The use of the upper confidence limit (95% confidence interval) addresses uncertainty about mean absorption due to sampling variability. This approach is reasonably conservative and could reduce the need to repeat studies.

112. Where results with large variability are used to compare relative absorption through rat and human skin in the 'Triple Pack' approach, a conservative method should be used. In some jurisdictions, 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 because of the different impact in calculation. 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

113. 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. Furthermore, clear statistical criteria to define outliers should be considered for removal and reported. Outliers should not be removed on statistical grounds alone; a plausible cause for the value being an outlier should be put forward, e.g. a membrane damaged during the experiment. If not, it should be considered part of normal experimental variation and not be left out. It should be noted that consideration of results treated as outliers should include spuriously low values as well as high ones.

7.5.5 Non-detects

114. According to OECD TGD 428, the analytical method including limit of detection and method validation should be reported. For radiolabelled dermal absorption studies, where study results are reported as a % of the applied dose, the impact of non-detect values might be minimal. If the impact of these values is small, it might be appropriate to use zero values for calculations. However, especially for cold methods with higher LOD/LOQ, the use of zero values might not be appropriate. Thus, the preferred approach for cold methods on how to take into account non-detects for the derivation of dermal absorption values from studies is as follows:

- Values below LOQ: use median of LOQ and LOD for calculation, since the estimated value is at an equal distance from LOD and LOQ, if the LOD is not reported, then ½ LOQ could also be used.
- Values below LOD: use LOD.

7.5.6 Rounding

115. There are two methods of rounding used for dermal absorption studies:

- In Europe, final dermal absorption values (i.e. after pro-rata or triple pack corrections) should be rounded to a maximum two significant figures. For example, 85.22% becomes 85%; 1.839% becomes 1.8%; and 0.268% becomes 0.27% (EFSA, 2017).
- In North American countries: 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. Values 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.

7.6 The 'wash-in' effect

116. 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.

117. 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).

118. As long as the skin depot following tape stripping 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.

119. 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.

120. EFSA (2017) offers a template (xls file) to support calculations on dermal absorption for in vitro studies according to the EU approach. Besides a practical example of the use, this template is published as supporting information.

PART 2: ESTIMATION OF DERMAL ABSORPTION IN THE ABSENCE OF SPECIFIC ROUTINE STUDIES CONDUCTED ACCORDING TO TG 427/428

8 INTRODUCTION

121. If available, specific experimental data should be used to determine the dermal absorption value for an active substance 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

122. In absence of experimental data, different default values are used under the different jurisdictions:

- In North American countries, 100% dermal absorption is used.
- In Europe, the following default values are recommended (EFSA, 2017):
  - 25% for concentrated products that are organic solvent-formulated(a) or in other(b) types of formulations;
  - 10% for concentrated products that are water-based/dispersed(c) or solid-formulated(d);
  - 70% for (in use) dilutions of organic solvent-formulated(a) or in other(b) types of formulations;
  - 50% for (in use) dilutions water-based/dispersed(c) or solid-formulated(d).

  These values are derived based on the statistical analysis performed on data from 420 human in vitro dermal absorption studies (GLP and OECD TG428-compliant) conducted with 246 active substances tested at different concentrations and in different formulation types (EFSA, 2017; dataset available at: https://zenodo.org/record/3378822#.XX0k2CgzbD5).)[1].

  For detailed information on formulation type categorisation, see Table B.2 in the EFSA Guidance (EFSA, 2017). Further, in order to decide whether a product is a concentrate or a dilution, in the EU, the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF) agreed on a pragmatic indication supplementary to EFSA (2017) based on an active ingredient concentration threshold of 5% (SANTE/2018/10591 rev.1.).

  A default value can be used in exposure calculations to cover a 'worst case' scenario. A dermal absorption value of 100% has to be assumed for corrosive formulations, in order to provide a high level of protection due to assumed damage of the skin (e.g. ECHA Technical Agreements for Biocides, 2017).

123. The physicochemical properties of a substance have a major impact on its dermal penetration. Thus, for example, it is widely assumed that large molecules and those with either a very low or a very high octanol-water partition coefficient (logPow), penetrate the skin to a much less extent than smaller molecules. In Europe, the analysis of human in vitro dermal absorption data with pesticides indicates both the molecular weight and the logPow are not good predictors for dermal absorption (EFSA, 2017). In some other jurisdictions (e.g. North America) information on physicochemical properties is used to reduce a default dermal absorption factor as part of a weight of evidence, including data from oral and dermal toxicity studies (Section 11.5), if available.

124. 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,

[1] Formulation types were grouped into 4 categories (organic-solvent, water-based/dispersed, solid and other) based on information on the chemical composition of the tested product, information on the phase in which the active substance is dissolved or emulsified/suspended and the expectable impact on dermal absorption, following the 'Manual on development and use of FAO and WHO specifications for pesticides' (FAO/WHO, 2016).

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.

125. In addition to the use of 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.

10 DERMAL ABSORPTION VALUE FOR EXPOSURES TO DRIED RESIDUES

126. For scenarios where workers, residents, and/or the general public may have contact with dried residues (e.g. agricultural post-application activities in treated crops by workers, children crawling or playing on lawn or surfaces treated with biocides, etc.), there is uncertainty when selecting a dermal absorption value from a study conducted with a liquid applied to the skin. In the above post-application scenarios, exposure would be to the dried diluted spray solution transferred to the skin from contact with the treated surface. This is different than for handler scenarios where the liquid solution is deposited on the skin. The different states (i.e. dry vs wet) and ways of loading chemicals on the skin seem to impact the penetration of pesticides through the skin. Based on current literature (Aggarwal et al, 2019; Clarke et al., 2018), dermal absorption from dried residues for a range of pesticides was higher than that from the concentrated product (nine liquid concentrates and one water dispersible granulate) and lower than that from the liquid diluted product.

127. Aggarwal et al. (2019) developed a methodology to determine the dermal absorption of dried residues using a polytetrafluorethylene-coated septum as surrogate for contaminated surfaces. From this septum the dried residues were transferred to the in vitro human skin assay. This methodology is not validated. It is suggested that dermal absorption of liquid spray dilutions would be higher than dermal absorption from dried residues, however, using dermal absorption of concentrates (nine liquid concentrates and one water dispersible granulate) would underestimate dermal absorption from dried residues. Due to the fact that some dermal absorption values of tested dried residues were close to dermal absorption values of tested liquid spray dilutions, no factor for refinement can be derived by the available literature. In addition, the database is very small (only ten formulations tested).

128. Furthermore, information on the influence of different loadings of dried residues on dermal absorption is limited. It is unknown to what extent dermal absorption would change with dose, and if it would be similar to the relationship seen in standard dermal absorption studies conducted with concentrate and diluted products. Thus, it should be noted that the tested dilution should cover the intended spray dilution.

129. Taking the above-mentioned considerations into account, unless chemical-specific data from a well conducted dried residue study is available, it is proposed that the appropriate dermal absorption value for exposures to dried residues should be from the respective in-use dilution if dermal absorption for the concentrate is lower. The value used should be based on a tested dilution which covers the spray dilution relevant for risk assessment. If dermal absorption for the concentrate is higher, then in this case the higher value should be used, unless there is a scientific justification (e.g. disruption of the skin barrier from the concentrate, which is not expected at the in-use dilution).

11 PREDICTION OF DERMAL ABSORPTION BY ALTERNATIVE APPROACHES

130. 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. A sound scientific basis for choosing a certain alternative method of prediction must always be provided. Companies should submit these 'theoretical considerations', taking into account that data should be provided to allow for increased transparency and to support of any theoretical considerations. 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.

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

11.1. Read-across

132. According to an OECD definition (OECD 2014), 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 or with chemicals with similar structures and physical chemical properties. 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. However, a read-across approach should only be used with caution; scientifically based justifications should take into account possible limitations and caveats

133. 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 were reliable

studies for substance A that were performed under GLP-like conditions and that are in compliance with OECD TG 427 and/or 428 (OECD, 2004a and 2004b).

134. 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.

135. 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, logPow, melting point, ionogenic state) should be considered as well as chemical structures, and these physicochemical properties might be of even higher importance. Recently, the OECD has published general guidance on read-across and the formation of chemical categories (OECD 2014). In addition, ECHA provides guidance on how to apply read-across for chemicals (ECHA, 2017a). Additional uncertainty arises from the possible impact of different co-formulants 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 co-formulants and the concentration of the test substance (a more detailed discussion of formulation effects is included in Section 7.2).

11.2. Quantitative structure-activity relationships (QSARs)

136. 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 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'. The EFSA Guidance on dermal absorption (2017) also provides some evaluation of literature on QSAR for skin absorption prediction. For example, new QSAR development includes neural network, nearest neighbour and Gaussian process models (Buist, 2016). Kneuer et al. (2018) aimed to investigate the applicability of in silico tools for the prediction of dermal absorption for pesticides. Based on a scientific literature search, models were selected for their potential usefulness regarding the prediction of dermal absorption of pesticides and their dilutions in water, or of pesticides from mixtures. After statistical analysis using a large dataset of in vitro dermal absorption studies (available at https://zenodo.org/record/3378822#.XWfi4-gzaUk), only one model was recommended to be further investigated regarding possible use in risk assessment of pesticides. However, it was noted that

improvement of predictivity is required and pesticide formulation influence was not taken into account comprehensively. Related research has been conducted by Eleftheriadou et a. (2019). Publicly available models for dermal absorption estimation were investigated based on data from human in vitro experiments on pesticides. The models generally failed to accurately predict experimental values. While overestimation is less critical for risk assessment, underprediction is not acceptable. Further, mixture information was not considered with the used models.

137. 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.

138. 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.

139. Many QSARs in that field predict the dermal permeability co-efficient KP 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). Nevertheless, while a few existing models predict the percentage dermal absorption, the applicability for pesticides is not ensured to the current state of knowledge (Kneuer et al., 2018).

140. 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.

11.3. Use of flux or permeability coefficient KP

141. As already described above, the percentage dermal absorption depends on several conditions like formulation, concentration, exposure duration, etc. Flux values are frequently reported, especially in the open literature, to describe dermal absorption under infinite dose testing conditions. No dose loss from abrasion, washing or volatilisation, or the absence of lag time might build some conservatism of the flux approach; nevertheless, it is difficult to quantify amount of conservatism in relation to other factors (e.g. dose). 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 as described hereafter.

142. The maximum flux at relevant exposure levels in milligrams per square centimetre per hour (mg/cm2/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.(e.g., 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).

143. The flux may be applicable in situations where the exposure is similar to 'infinite exposure', such as exposure to chemicals, such as biocides in swimming pools or from 'leave-on' topically applied products. As an exceptional example, a transdermal flux rate was derived during biocidal active substance evaluation of propan-2-ol due to the expectation of significant volatilisation during exposure situations and subsequently the reduction of substance available for dermal absorption during exposure (e.g. Assessment Report of propan-2-ol in the context of Reg. (EU) No 528/2012, product-type 2, 2015). Furthermore, 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 (e.g. such depots can be particularly significant for highly lipophilic substances (in stratum corneum) and substances with strong protein binding). Inter alia, other shortcomings might question the applicability of Kp / Jmax for pesticides:

- Dermal Jmax algorithms are based on empirical dermal absorption datasets –the applicability outside original training sets is unclear.
- There is a lack of validation outside the 'chemical space' of cosmetic ingredients, fragrances and fragrance-like ingredients.
- Dermal Jmax may not be advisable for substances that are large (MW >350 Da), highly lipophilic (log Kow >6) and/or have a low water solubility (<0.1 mg/L), at least without further methodological analysis.
- Dermal Jmax is not advisable if substance is in formulation with skin penetration enhancers which is almost always the case for pesticide formulations.

For substances that are very small (MW <100 Da) and/or highly volatile (>100 Pa), the Dermal Jmax Approach tends to over-predict absorption.

144. In an in vitro dermal absorption experiment under infinite-dose steady-state conditions, KP 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, KP can be derived from the non-linear part of the permeation kinetics).

145. The product of KP 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 KP were applicable in a certain regulatory situation (such as 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).

11.4. 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 (refer also to Kneuer et. al, 2018). However, there are models that could be helpful to conclude from KP 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).

11.5. Comparison of toxicity data from oral and dermal studies

149. Oral and dermal toxicity studies with repeated administration are usually not considered appropriate to estimate dermal absorption of a substance (EFSA, 2017), but, in some jurisdictions (North American countries), might be used in exceptional cases in a weight-of-evidence if:

- 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 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 Cmax-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 toxicity study, a NOAEL at the limit dose (1000 mg/kg/day) used in this extrapolation method may be considered a conservative estimate of dermal absorption, although this value deals with toxicity. 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. However, for regulatory purposes usually no oral and dermal toxicity studies with repeated administration performed with formulations are available. Thus, the influence of the formulation is disregarded so that these data do not provide an accurate estimate of dermal absorption for an active substance in formulations. In addition, other circumstances (e.g. different animal strains, study duration,

possible first pass effects etc.) may conflict with the use of these data. Generally, in Europe (EFSA, 2017, 2012) it is not recommended to derive the dermal absorption of a compound by comparing the toxicity produced at different dose levels via the oral and dermal routes.

151. 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.

152. 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).

153. 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.

154. 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.

155. 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. However, in North America countries, when this is the only available data, it may be used.

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

11.6.1. In vivo ADME studies

156. 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. In addition, there are usually no ADME studies for formulations that include co-formulants which are often modifying dermal absorption. For these reasons, estimates should be applicable in only a limited range of circumstances after careful consideration of doses and vehicle used in the ADME studies, where bile-cannulation was also performed.

11.6.2. The 'mass balance' approach

157. 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.

158. 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.
- 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.

159. 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.

11.6.3. Human in vivo dermal absorption studies

160. In some jurisdictions, 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). In jurisdictions that may allow the use of such studies they must be found to be scientifically sound and conducted ethically (e.g., US may require review by a dedicated committee).

11.6.4. Human biomonitoring studies

161. Although biomonitoring data might be useful for operator or worker exposure assessment, it is considered not suitable to extrapolate dermal absorption from such a study, as the dose – the amount of chemical in contact with the skin – is not accurately known. As human biomonitoring accounts for all sources (workplace exposure, air, water, diet, consumer products etc.) and all routes of uptake, human biomonitoring data as such are not suitable for the assessment of a specific dermal exposure of a substance when other routes of and sources of exposure are involved.

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ANNEX I - DEFINITIONS

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

Applied dose: mass of test preparation containing a specified mass of test substance applied per cm2 of skin.

Dermal absorption: The movement of a chemical from the outer surface of the skin into the circulatory system, eventually leading to systemic exposure towards the chemical (dermal bioavailability) and its metabolites. Also called percutaneous absorption.

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

Dermal penetration: The movement of a chemical from the outer surface of the skin into the epidermis and dermis, but not necessarily into the circulatory system.

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.

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.

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 (µg/cm2/h).

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.

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

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

Permeability coefficient (KP): 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.

TEWL: Transepidermal water loss; the loss of water that passes through the epidermis from the inner body to the surrounding atmosphere; used for characterisation of the skin water barrier function.