Monograph Report

No 20

Percutaneous Absorption

August 1993

ISSN-0773-6347-20

Monograph No. 20

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Brussels, August 1993

ECETOC Monograph No. 20

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CONTENTS

SUMMARY

Factors influencing the percutaneous absorption of chemicals have been reviewed. Descriptions of how the physico-chemical properties of a chemical, the nature of the skin exposure to the chemical, vehicles and variations in the physiological nature of the skin-site influence the absorption process are presented. It has not been possible to reach firm conclusions on how to make precise quantitative predictions about percutaneous absorption from such factors.

A variety of methods for measuring percutaneous absorption are discussed. It is concluded that the most reliable data for predicting absorption through human skin come from human volunteer experiments. Alternative approaches using animal models *in vivo* or *in vitro* techniques using animal and human skin are presented. The limitations and advantages of these various techniques are described with sufficient detail to allow the generation of scientifically sound absorption data. It is considered that data generated from *in vitro* percutaneous absorption studies (using *ex vivo* skin) should be accepted by regulatory authorities.

Recommendations are made for the presentation of data from percutaneous absorption studies. Details are given of the ability of animal skins to predict human percutaneous absorption; data suggest that pig and monkey skins are the best models for human skin permeability.

Techniques which may be used to quantify human dermal exposure are presented.

Assessment of hazard from dermal exposure can be made by considering available No Observed Effect Level (NOEL) as determined in toxicology studies and human systemic exposure data. As a first step it is reasonable to assume 100% dermal absorption and that systemic exposure is equal to dermal exposure. If this indicates there is no hazard no further estimates are required. If the calculation suggests 100% absorption and this would be hazardous, the actual degree of percutaneous absorption, ideally determined in human volunteers, should be quantified and the hazard reassessed. If this still shows a hazard exists, risk management techniques to eliminate or minimise dermal exposure will be needed.

SECTION 1. INTRODUCTION

It is recognised that chemicals can be absorbed through the skin. Such absorption can be desirable such as in topical and transdermal delivery of drugs (Shaw *et al*, 1976; Shrewsbury *et al*, 1980) or undesirable with dangerous and even fatal consequences (Davies *et al*, 1979).

This document reviews current understanding of the movement of chemicals through skin, i.e. percutaneous absorption. Percutaneous absorption studies are not intended to examine the subsequent distribution, metabolism and excretion of the absorbed chemical, although this information may be necessary to understand the consequences of percutaneous absorption.

To elicit a systemic effect, a chemical applied to the skin must pass through the outermost layer of the skin, the *stratum corneum*, and into the viable epidermis and superficial dermis. Normally, the rate limiting step in this process is diffusion through the *stratum corneum*, after which movement across the epidermis and superficial dermis to the vast capillary network beneath the dermo-epidermal junction is rapid. Percutaneous absorption is, therefore, defined as the movement of chemicals from the outer surface of the skin to the systemic (circulatory) system. The process of percutaneous absorption is illustrated in diagrammatic form in Figure 1 and described in Section 3.

ECETOC established a Task Force to consider the available knowledge in this area and how the hazard to man of chemicals absorbed by this exposure route can be assessed. Its Terms of Reference were:

- to review *in vitro* and *in vivo* methods for measuring percutaneous absorption,
- to discuss their relevance, interpretation and use of the findings when forming interspecies comparisons,
- to review methods for measurement and assessment of dermal exposure,
- to discuss the relevance of the above information when identifying the potential for percutaneous absorption and conducting hazard/risk assessment for industrial chemicals.

The definition of terms used in this Monograph are given in Appendix A; those relevant to hazard and risk have been taken from an EEC publication (EEC, 1990).

SECTION 2. BACKGROUND

Assessments of percutaneous absorption are now routinely done in many laboratories throughout the world. The majority of studies have used radiolabelled test substances. These studies are performed, for example, to assess dermal pharmacokinetics and to identify potential hazards resulting from exposure to chemicals in the workplace, from migration of chemicals present in cosmetics or from migration of chemicals present as process additives or contaminants in textiles, e.g. dyes, detergents.

Dermal exposure can be defined as the amount of a chemical in contact with a unit area of skin and for a defined time period. Exposure can be to pure chemicals or preparations containing them; such differences can affect the absorption process. Contact with skin and subsequent absorption will lead to systemic exposure which may be of clinical significance.

A number of guidelines to quantify percutaneous absorption of agrochemicals have been published, e.g. the EPA guideline (Farber and Zendzian, 1990; Zendzian, 1991) and the British Agrochemical Association (BAA) guideline which has been accepted by the UK Ministry of Agriculture and Fisheries and Food (MAFF). These BAA protocols have been submitted for consideration to the OECD (BAA, 1989). At present there is no single, accepted approach to the generation of these data.

Existing data on the absorption of chemicals through skin have been obtained using a variety of *in vivo* and *in vitro* methods. The *in vivo* methods have used human volunteers (Feldmann and Maibach, 1974) and a range of animal species (Knaak *et al*, 1984; Grissom *et al*, 1985). The percutaneous absorption of chemicals has also been extensively studied using *in vitro* techniques with human (Dugard *et al*, 1984) and animal skins (Bronaugh *et al*, 1982a,b; Scott and Corrigan, 1990) as well as a variety of synthetic membranes (Ridout *et al*, 1990).

Examination of these test methods shows no consistency in dose, vehicle or exposure time used. The results are also expressed differently, e.g. in terms of percentage of the applied dose absorbed, absorption rates or permeability coefficients, so it is difficult or impossible, to compare results obtained with different test procedures. In addition, the data derived from *in vivo* (Bartek *et al*, 1972) and *in vitro* (Scott *et al*, 1986a) studies using animal skin have shown that there are wide interspecies variations in skin permeability, making many of these data difficult to use for predicting human percutaneous absorption.

When a potential for percutaneous absorption has been identified a hazard assessment requires an assessment of the potential for human exposure, based on the intrinsic characteristics of the material and preparations, matrices or articles in which it is found. It is only when data from both the potential exposure and percutaneous absorption are available that it is feasible to quantify the hazard to man of dermal exposure to chemicals.

Once a hazard has been defined the probability that sufficient exposure would occur should be established to assess the risk. The outcome of such a risk assessment will allow further decisions to be made to decrease human dermal exposure (the risk management process).

The factors affecting percutaneous absorption of chemicals are discussed and the various methods which can be used to measure absorption are reviewed. The ability of these methods to predict *in vivo* human absorption is considered. Recommendations for the presentation of absorption data to facilitate intercompound comparisons are given. Techniques which may be used to quantify human dermal exposure are presented. The use of these and percutaneous absorption data in hazard and risk assessment is described.

SECTION 3. FACTORS INFLUENCING PERCUTANEOUS ABSORPTION

3.1. INTRODUCTION: THE PROCESS OF PERCUTANEOUS ABSORPTION

The following description of percutaneous absorption is provided to complement Figure 1 and is based on current understanding. A number of important steps are acknowledged (see Figure 1 and numbers in text). This description is a general guide rather than a comprehensive explanation: the latter is outside the remit of the Task Force although suitable references are given as sources of further information.

For a chemical to be absorbed through the skin, the chemical (which will now be referred to as the penetrant) must first diffuse to the outer surface of the skin (2), the *stratum corneum* (SC). Chemicals which are not in solution on the surface of the SC, such as crystals or suspended penetrants in a vehicle/formulation, must first undergo a process of dissolution (1) before dissolving in the outer SC. The extent of the movement of the penetrant into the SC is influenced by its solubility in the SC. The penetrant will partition (3) between the two phases i.e. dissolved penetrant on the surface of the SC or in solution in a vehicle/formulation and the SC and establish an equilibrium (thermodynamic activity equal in both phases). Once in the SC the penetrant will diffuse (4) from the high concentration in the outer SC down to the lower layers of the SC where the concentration is less. Some of the penetrant entering the SC might bind (5) to SC components and not be available to diffuse further.

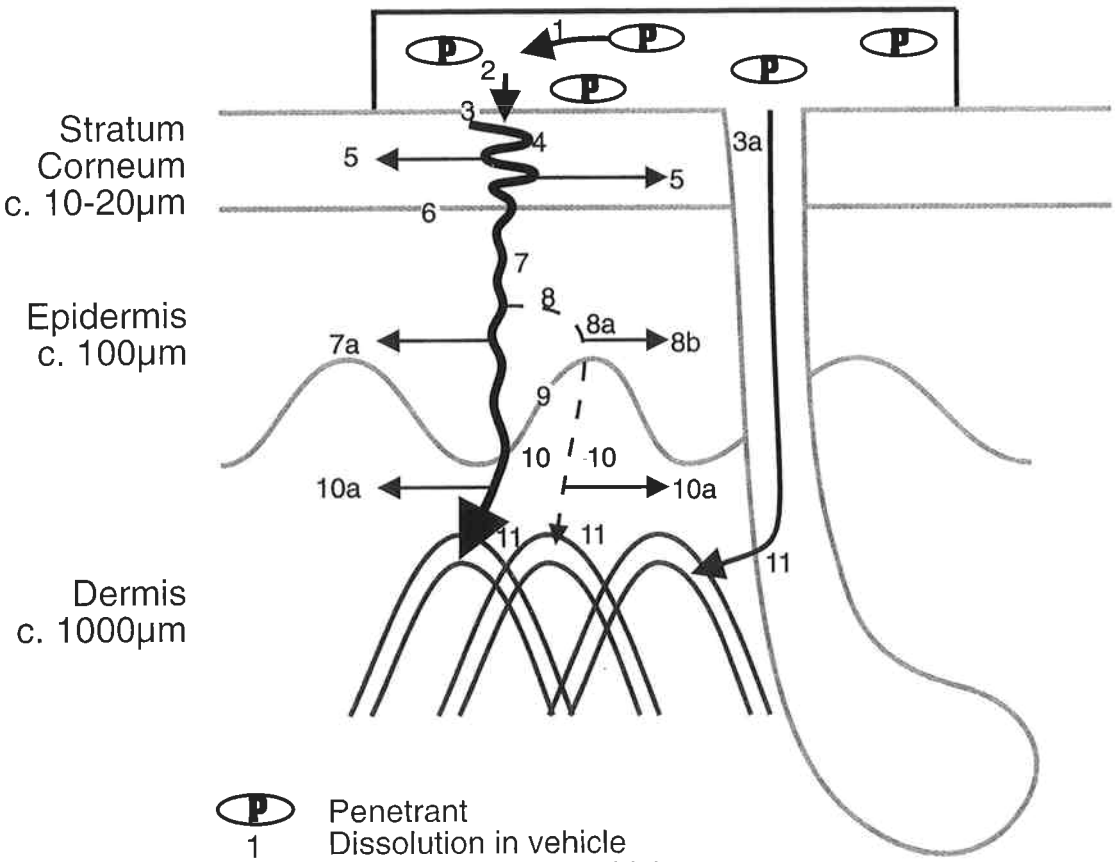
Although the SC is the primary barrier to the absorption process, penetrants might diffuse through potential rapid routes (3A), such as the hair follicles and sweat glands, which by-pass this barrier.

At the base of the SC the penetrant must undergo another partition step (6) from the SC into the viable epidermis. Diffusion through this layer (7) towards the capillary network immediately below the dermo-epidermal junction is relatively rapid. During this phase, the penetrant might also be prevented from diffusing further by binding (7A) or might be metabolised (8). The metabolite(s) can also diffuse to the capillary network (8A) or be bound in the epidermis (8B). At the base of the epidermis the diffusing penetrants (parent chemicals and any metabolites will partition between the epidermis and the dermis (9) before entering the systemic circulation (11).

Fuller details of this process may be found in Schaefer *et al* (1982) and Barry (1983).

The extent of percutaneous absorption of a chemical depends on a series of factors which are related to the chemical itself, to the exposure conditions, to the formulation or vehicle containing the chemical and to the characteristics of the skin. In the following sections, these factors are addressed in greater detail. The influence of the species of animal on skin permeability is discussed in Section 6.

Figure 1 The Process of Percutaneous Absorption



P	Penetrant
1	Dissolution in vehicle
2	Diffusion through vehicle
3	Partition from vehicle to outer layer of stratum corneum
3a	Diffusion/Partition into shunt pathways
4	Diffusion through stratum corneum
5	Binding/Reservoir formation in stratum corneum
6	Partition from stratum corneum into epidermis
7	Diffusion across epidermis
7a	Binding in epidermis
8	Metabolism of parent to form metabolites
8a	Metabolite(s) diffusion through epidermis
8b	Metabolite(s) binding in epidermis
9	Pertition between epidermis and upper dermis
10	Diffusion across upper dermis
10a	Binding in dermis
11	Uptake into capillary network

3.2. FACTORS INFLUENCING PERCUTANEOUS ABSORPTION

3.2.1. Physico-chemical properties of the penetrant

The absorption, i.e. diffusion, of a chemical through the *stratum corneum* depends on chemical-specific factors such as molecular weight, water and lipid solubility, polarity and state of ionisation (Mathias,

1983). In general, small molecules which are both lipid- and water-soluble are the most readily absorbed through the essentially lipophilic environment of the *stratum corneum* and hydrophilic environment of the epidermis. Relatively large differences in molecular weight are required to alter diffusivity significantly since the diffusion coefficient is theoretically inversely proportional to the cube root of molecular weight (Zatz, 1983). It is generally recognised that the lipophilicity of a chemical, as measured by the log octanol/water partition coefficient value, log P_{ow}, can influence absorption. The absorption of chemicals with log P_{ow} values less than -1 is not markedly affected by changes in this value and these chemicals are usually poorly absorbed. Above this value and up to log P_{ow} values of approximately +3.5 absorption tends to increase as the log P_{ow} increases. Maximum absorption is often associated with values of log P_{ow} between +1 and +2.

3.2.2. Duration of Exposure

Percutaneous absorption begins when a chemical first contacts the skin, but there may be a delay of less than 30 minutes (Rougier *et al*, 1985) to many hours (Howes and Black, 1975) before the chemical enters the systemic circulation. If the chemical is not washed off the skin, the concentration on the skin may decrease asymptotically to zero due to absorption or loss from the surface by, for example, desquamation or evaporation. If the chemical is washed off, residual material in the epidermis (ie. *stratum corneum*) may continue to be absorbed for some time.

In the experimental situation and in the clinical administration of systemic medication by dermal patches, the application duration may be fairly clear, though the duration of absorption may be less clear. In topical treatments where the application is not washed off and in accidental exposure when the fact or time of first contact may not be recognised, the application duration may not be known.

Generally, the longer a chemical is on the skin, the greater will be the total absorption, but the difficulty in defining and determining the exposure duration has prevented the identification of a simple relationship between exposure duration and total absorption (Barry, 1983) with the notable exception of Rougier *et al* (1985).

3.2.3. Frequency of Exposure

This factor is important in determining the magnitude of dermal and systemic exposure over prolonged time periods (Wester *et al*, 1977; Bucks *et al*, 1985; 1989). Frequency of exposure is highly dependent upon the nature of activities which result in contact with a compound, e.g. soil excavation, gardening, chemical manufacturing, application of cosmetics or personal care products (Driver *et al*, 1989).

3.2.4. Dermal Area Dose

An important determinant of the absorption rate, i.e. the substance flux from the skin surface into the systemic circulation, is the dermal area dose (amount of test chemical applied per cm^2 of skin). When a vehicle is used, or a chemical is in a formulation, then the area-dose depends on the amount of vehicle/formulation applied per cm^2 of skin and the concentration of the chemical.

When applying constant amounts of a non-volatile solution which contains the test chemical in varying concentrations to the skin surface for a constant exposure time, the penetration rate increases with increasing concentration of the test chemical in the vehicle. Once the solution becomes saturated the absorption rate does not increase further (Zatz, 1983; Wester and Maibach, 1989c). Nevertheless, where super saturation results from evaporation of vehicle components, the absorption rate may increase further (Davies and Hadgraft, 1991). The rate of absorption can be higher from a dilute solution in one vehicle than from a more concentrated solution in a different vehicle (Dugard and Scott, 1986). This may be caused by vehicle-dependent partitioning into the *stratum corneum* (cf. 3.2.5). The relationship between the rate of percutaneous absorption and the concentration of chemical is not necessarily linear. Increasing the concentration of a corticosteroid in an ointment vehicle (amount per mg of vehicle, but not definitely the concentration in solution) 50-fold from 0.1 to 5% only increased the percutaneous flux ca. 4-fold. This suggests that the dissolution of a suspended penetrant in the vehicle might also influence the absorption process (Taeuber and Herz-Huebner, 1977).

Walker *et al* (1991) investigated the significance of vehicle thickness using a corticosteroid cream by varying the application dose from 1 to 40mg of formulation per cm^2 (corresponding to average film thicknesses of 10 to 400 µm). The percentage of applied dose of the corticosteroid absorbed through isolated human epidermis in 72 hours decreased with thickness. It was found that the percutaneous penetration rates increased, as the dose was increased up to $5mg/cm^2$ of skin and then remained constant at skin area doses equal to and higher than $5mg/cm^2$. Experience with dermato-pharmaceuticals has shown that the skin area-dose during therapeutic use is 2 to 4mg preparation/cm^2 corresponding to film layers of 20 to 40µm thickness (Lutz and Weirich, 1975). The dermal doses of general purpose cosmetic creams has been estimated to 1mg product/cm^2 (cf. Appendix B).

3.2.5. Vehicle/*stratum corneum* Partition Coefficient

The vehicle/*stratum corneum* partition coefficient is an important factor in determining the rate of penetration of a chemical (Scheuplein and Blank, 1971). This coefficient describes the relative affinity of a chemical for the vehicle in which it is applied and the *stratum corneum* (Suskind, 1977). It is a function of both the vehicle and the skin. The more soluble the penetrant in the vehicle, the more likely it is to be retained within the vehicle (Baker, 1979). Greater solubility in the *stratum corneum* and lower solubility in the vehicle promotes penetration (Nater and de Groot, 1985).

Special considerations may be necessary when evaluating the relative affinity of a compound for the skin with some solid vehicles. If the compounds are associated with particles (e.g. granules, house dust, soil, partitioning from the particle surface to the skin) desorption and volatilisation from the particle surface to the ambient air or to aqueous media (e.g. water, sweat) can be critically important in determining the compound's bioavailability (Driver *et al*, 1989).

3.2.6. pH Changes

Changes in the pH of the applied solution can influence absorption by altering the degree of ionisation of the compound; ionisation decreases the rate of absorption. In addition, the integrity of the absorption barrier could be affected if the *stratum corneum* is damaged by a very high or very low pH, resulting in increased penetration (Grasso and Lansdown, 1972; Zatz, 1983).

3.2.7. Anatomical Site

The *stratum corneum* is not of a uniform thickness or chemical composition, it may vary over areas of the human body, so that different absorption rates occur depending upon the skin site in contact with the chemical. In general, absorption will be greater where there is a thin *stratum corneum*, e.g. on the genitalia, and lower where the *stratum corneum* is thick as on the palms and soles. The *stratum corneum* covering the palms and soles is not as impermeable as might be expected from its thickness because its cells are structurally different from the flattened, tightly-bound cells which make up the *stratum corneum* in other areas of the body (Kligman, 1983). Thus, although the *stratum corneum* on the palms is 40 times as thick as on the forearm, it is not 40 times less permeable. Table 1 shows how variation in permeability can affect absorption rates in selected areas of the human body (Guy and Maibach, 1989).

TABLE 1 **Penetration Indices (Skin permeability relative to the permeability of the arm skin) for 5 Anatomic Sites Assessed Using Hydrocortisone Skin Penetration Data and Pesticide (Malathion and Parathion) Absorption Results [from Guy and Maibach (1989)]**

Site	% body area (adults)	Penetration index based on	
		Hydrocortisone data (Feldmann and Maibach, 1967)	Pesticide data (Maibach *et al*, 1971)
Genitals	1	40	12
Arms	18	1	1
Legs	36	0.5	1
Trunk	36	2.5	3
Head	9	5	4

3.2.8. Temperature of Skin

An increase in skin temperature usually enhances penetration since diffusion is temperature-dependent (Kligman, 1983; Hurley, 1985; Nater and de Groot, 1985). Skin temperature may be increased by the temperature of water used for bathing, the use of bath oils or body lotions, or simply by wearing clothing (Zatz, 1983; Brown *et al*, 1984; US-EPA, 1984a).

3.2.9. Hydration of the *stratum corneum*

Under normal environmental conditions the *stratum corneum* contains between 10 and 20% water by weight. The potential for hydration is evident from *in vitro* studies which show that the *stratum corneum* can take up 6 times its weight in water when immersed in 37°C water (Baker, 1979).

The use of materials, e.g. bath oils and skin moisturisers, may increase the water content of the *stratum corneum*. For example, the use of skin moisturisers leads to an overall increase in the water content of *stratum corneum*. This increase was not uniform across the tissue and a 100% increase was found in the layers nearest the surface (Wu *et al*, 1983). Hydration of the *stratum corneum* by high ambient humidity or wetting of the skin, as during bathing, swells the outer cell layer of the *stratum corneum* making it more permeable (Kligman, 1983; Hurley, 1985). The changes in the penetration rate produced by changes in hydration vary depending on the physico-chemical properties of the penetrants (Zatz, 1983).