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PERCUTANEOUS ABSORPTION

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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.

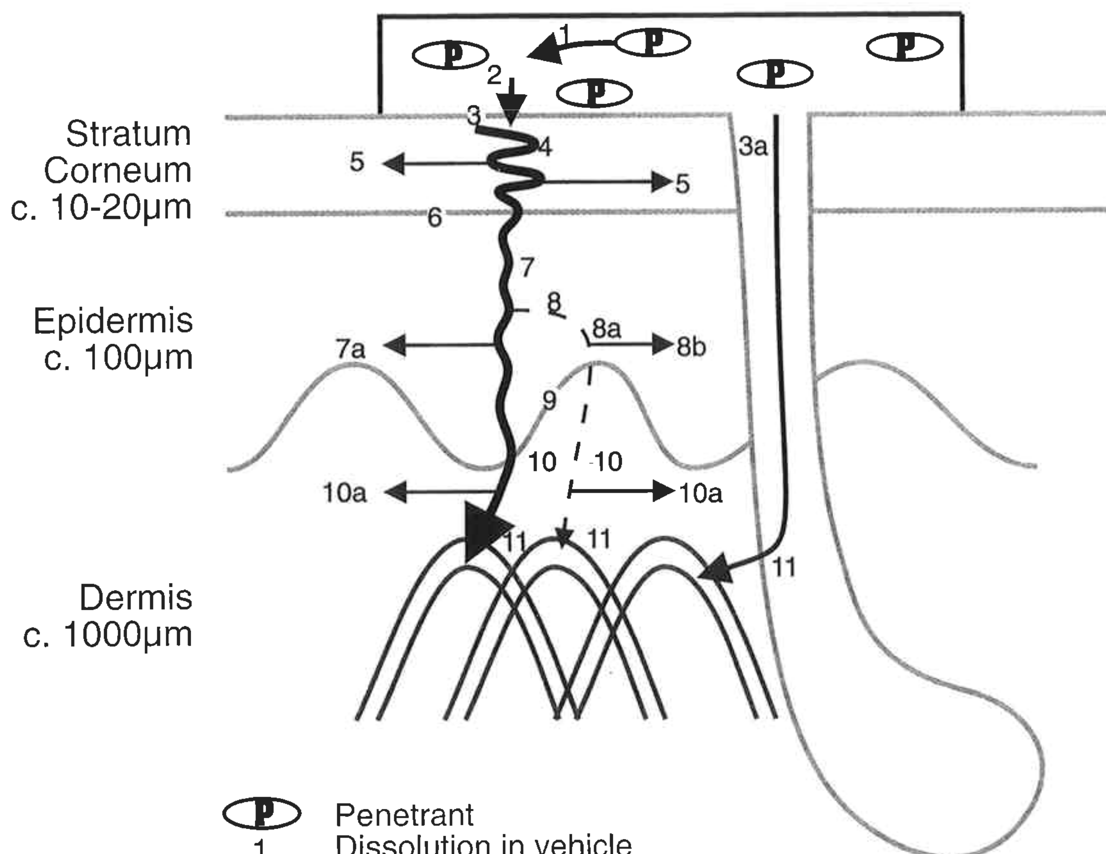
Although the SC is the primary barrier to the absorption process, penetrants might diffuse through potential rapid routes (3A), such as the hair follicles and sweat glands, which by-pass this barrier.

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).

3.2.10. Damage to the *stratum corneum*

Damage to the *stratum corneum* greatly decreases its effectiveness as a barrier. Solvents, acids, bases, denaturants, surfactants and other chemicals which may damage it will increase its permeability (Kligman, 1983). Lipid solvents can increase the penetration rate by removing structurally essential lipids from the *stratum corneum.* Because of its protein nature, it is vulnerable to hydrolysis by acids and bases. Soaps and detergents at high concentrations may solubilise its lipids and proteins (Anon, 1980).

Dehydration of the skin caused by cold, dry environmental conditions can lead to splitting of the *stratum corneum* (chapping) if the water content falls below 10%. The damaged cells then allow irritants such as soaps to penetrate more readily (Baker, 1979). Penetration rates may be increased by orders of magnitude by damage to the barrier layers (Zatz, 1983). Skin conditions such as physical damage, sunburn, psoriasis, dermatitis and eczema, also damage the barrier and enhance permeability (Baker, 1979; Brown *et al*, 1984; Nater and de Groot, 1985).

3.2.11. Other Factors influencing Absorption

No important differences in skin permeability have been reported between the sexes (Rougier *et al*, 1988) but behavioural differences may be significant, e.g. females tend to bathe more than males and use moisturising creams, bath oils, body lotions, etc., which can have an occlusive effect and may also raise the temperature of the *stratum corneum* (Baker, 1979). Minor racial differences in skin permeability have been reported. Age seems to be a factor. Permeability of the skin in pre-term infants is greater than in full-term infants (Rasmussen, 1978; West *et al*, 1981; Wester and Maibach, 1982). In the elderly (>65 years) reduced percutaneous absorption was found with relatively hydrophilic compounds (Roskos *et al*, 1989). No firm conclusions about the influence of such factors can be given because of the paucity of the human data.

Binding of penetrants to components of the *stratum corneum* might also influence absorption, either increasing the amount of time required for penetration or removing the substance from the skin when the *stratum corneum* cells are shed. Relevant data on this are very limited and inconclusive (Bucks *et al*, 1989).

Metabolism in the skin could affect percutaneous absorption. Although this would probably be of minor significance, a noticeable effect might be observed with slowly absorbed substances (Bronaugh, 1989; Bronaugh *et al*, 1989a).

SECTION 4. METHODS FOR MEASURING PERCUTANEOUS ABSORPTION

The key questions to be addressed in percutaneous absorption studies are quantification of the amount of chemical which penetrates the skin barrier and reaches the circulatory system and how this can be measured. Absorption can be assessed by the measurement of concentration of chemical in body fluids, tissues or excreta for *in vivo* studies or in the receptor solution (cf. section 4.2.) for *in vitro* studies (Kemppainen and Reifenrath, 1990).

Radiolabelled chemicals are often used in such studies. The quantification of absorption may not be specific because the chemical nature of the penetrant is normally not known. The radiochemical purity of the test chemical must be high to avoid overestimation of percutaneous absorption caused by presence of labelled impurities. The specific radioactivity of the labelled chemical must also be as high as practically possible to optimise the sensitivity of the analysis (Van Lier, 1985).

The objectives of any percutaneous absorption study must first be considered. Simple dermal absorption measurements might be adequate. Alternatively a more sophisticated experiment should be attempted to mimic, for example, accidental human exposure by spillage of a chemical under a glove will need to be reflected in the final study design which must also consider:

- the choice of solvent or formulation and the concentration of the test chemical;
- the method of application to the skin which may be direct as a solution or suspension or indirect from a patch of material treated with the chemical;
- the duration of exposure and whether the dosing site is covered (occluded) or open to the atmosphere (non-occluded).

All of these factors can have a significant effect on absorption (cf. Section 3).

Volatile chemicals pose a further complication since their availability for absorption will be reduced by evaporation. The inclusion of skin washing and drying may further mimic human activity but this can also affect the extent of absorption (Wester and Maibach, 1985b; Kao, 1990).

The use of *in vivo* and *in vitro* methods, and other approaches employed at present to assess the potential for percutaneous penetration, are subsequently described and summarised.

Guidelines for the conduct of these studies are given in Appendices C and D.

4.1. *IN VIVO* STUDIES

4.1.1. Study Design

The test chemical is applied to a designated area of skin in solvent or formulation or to a patch of material which is then placed at a predetermined site in direct contact with skin for a defined period. Body fluids, tissue or excreta are collected at predefined intervals and the quantity of chemical and/or metabolite in the samples is measured by a suitable analytical procedure. Complementary experiments with parenteral or oral dosing may be required to provide information on the retention of the chemical within the body and the routes and rates of elimination. Knowledge of the metabolic fate of the chemical is especially important when unlabelled compounds are used to ensure that the correct analytical procedures are followed. The analytical method of choice must be appropriately sensitive since percutaneous absorption is often low. If little absorption is expected, quantification may be optimised by treating large areas of skin, e.g. the whole of the back for man.

More details on *in vivo* studies are given in Appendix C.

4.1.2. Human Volunteer Studies

These studies provide definitive data for the assessment of the absorption of chemicals through human skin but for technical and ethical reasons their use is limited and their conduct is closely regulated (WMA, 1982; Declaration of Helsinki, 1983). A study protocol with supporting toxicological data must be submitted to an ethical committee and approval obtained before any study commences. The use of radiolabelled compounds for human studies is subject to yet further regulation (ICRP, 1977, 1979; NRPB, 1988).

For the development of pharmaceutical products the use of human volunteers is considered to be the only relevant approach to generate data of precise relevance to man.

The most common study design utilises the analysis of urine after application of the test chemical to the skin surface in a vehicle (Feldmann and Maibach, 1974), a formulation (Wester *et al*, 1984; Bonsall and Goose, 1986) or as patches of material treated with the chemical (Newton and Norris, 1981; Kolmodin-Hedman *et al*, 1983). To estimate the dermal dose absorbed the results from the dermal experiment are corrected for the proportion of chemical excreted in urine after parenteral or oral administration. Parenteral administration is the most appropriate route since the chemical is 100% systemically available. Parenteral administration is, however, very rarely possible for ethical reasons.

Human volunteer studies are most relevant for human risk assessment and they provide data for human exposure monitoring.

The disadvantages are that they give rise to ethical problems, they only measure absorption indirectly, metabolism data are required, there are analytical problems (low absorption, restricted use of radiochemicals) and the dermal and oral pharmacokinetics may not be the same due to differences in disposition and metabolism.

4.1.3. Animal Studies

The use of animal models provides physiologically and metabolically intact systems with which to study absorption. Data from such studies will overestimate human percutaneous absorption but are useful as screens in pharmaceutical product development and to generate data for hazard assessment. Many species have been used including mice (Grissom *et al,* 1985; Marco *et al*, 1985); rats (Bartek *et al*, 1972; Knaak *et al*, 1980, 1984; Bronaugh *et al*, 1982a; Shah and Guthrie, 1983; Marco *et al*, 1985; Shah and Hall, 1990); guinea pigs (Howes and Black, 1975; Chow *et al*, 1978; Andersen *et al*, 1980); rabbits (Bartek *et al*, 1972; Bartek and La Budde, 1975); pigs (Bartek *et al*, 1972; Bartek and La Budde, 1975; Reifenrath *et al*, 1984a, b); and monkeys (Nacht *et al*, 1981; van Lier, 1985) as well as more specialised breeds of hairless mice (Susten *et al*, 1986), rats (Rougier *et al*, 1987) and dogs (Hunziker *et al*, 1978; Reifenrath *et al*, 1984b) or skin grafted animals (Reifenrath *et al*, 1984a). A general summary of these protocol designs is given below and in Appendix C.

In addition to the indirect method of assessing percutaneous absorption by monitoring chemical and/or metabolite eliminated in urine or faeces (cf. 4.1.2.) a mass-balance study is possible in which radioactivity is quantified in excreta, in blood, at the dose-site, in the remaining carcass of the animal and in expired air (Marco *et al*, 1985). Measurement of chemical in any solutions which have been used to wash the application site, or in the *stratum corneum* which has been removed by tape stripping (a technique by which *stratum corneum* is removed by the repeated application and removal of adhesive tape) or associated with the application site cover, is also required.

The major advantages of animal studies are:

- test animals are readily available, easy to handle and to maintain;
- radiochemicals can be used without restriction;
- animals are physiologically and metabolically intact;
- animals can be used in other toxicology/metabolism studies on the same substances;
- all chemical types (pharmaceuticals, agrochemicals, industrial chemicals) can be studied;
- new chemicals, of which toxicity or other properties are not completely defined can be examined and
- the techniques using animals are recognised as of value by regulatory authorities.

Disadvantages of animal studies are:

- they do not always provide an accurate indication of human skin permeability;
- they use living animals;
- the dose site has to be protected to prevent removal of chemical by normal grooming and
- the determination of early absorption phase is difficult.

4.1.4. Comparison of Regulatory Guideline Protocols for *in vivo* Dermal Absorption Studies with Animals

Both the US Environmental Protection Agency (Zendzian, 1991) and the British Agrochemical Association (BAA, 1989) have produced protocols intended to provide information for the registration of agrochemicals. The main features of these experiments are described in Table 2. The designs are similar but the EPA protocol requires measurement of absorption after varying periods of exposures up to 24h (following a single application), the BAA protocol measures absorption after an 8h exposure to the chemical (a typical work day). A major disadvantage of the EPA approach is the high number (96) of test animals required.

4.2. IN-VITRO STUDIES

4.2.1. Relevance of *in vitro* Percutaneous Absorption Studies

Percutaneous absorption is a passive diffusion process (Bronaugh *et al*, 1982a; Dugard, 1987) and the permeability properties of the *stratum corneum* are unchanged by its removal from the body (Bronaugh *et al*, 1982b). Evidence from the literature indicates that *in vitro* percutaneous absorption data can/do predict *in vivo* absorption. Although all the data are not presented here, sufficient evidence exists to give confidence, that when *in vitro* methods are used, (cf. Appendix D) the available techniques provide data which predict *in vivo* absorption.

Some examples of the available human data are presented in Table 3 and some animal skin data in Table 4.

An early study (Franz, 1985) compared the *in vitro* absorption of 12 compounds through human skin with previously published (Feldmann and Maibach, 1974) *in vivo* data from human volunteer studies. There was such a good correlation between the human *in vivo* data and the *in vitro* data that this paper stimulated further comparisons. Other authors have generally reported that the *in vitro* technique was able to accurately predict *in vivo* absorption through human skin (see ratios between *in vivo* and *in vitro* data in Table 3). This ability of *in vitro* techniques to predict *in vivo* absorption has now been proven for a range of chemicals with different physico-chemical properties.

TABLE 2 A COMPARISON OF REGULATORY GUIDELINE PROTOCOLS FOR *IN VIVO* DERMAL ABSORPTION STUDIES ON AGROCHEMICALS

	Origin of Guideline	
	US-EPA (1984b)*	BAA (1989)
Stated objective	The determination of dermal absorption in relation to dose and time of exposure.	Determine absorption as a result of an 8h dermal exposure (dose site washed at 8h).
Animals sex strain number	Young adult rats (225-250g) male as used in tox./metab. studies 4 per time point.	young adult rats (8-14 weeks) within hair resting phase (dormant telogen) one sex as used in tox./metab. studies 5 per time point.
Dose level	Minimum 3, preferably 4 log intervals (e.g. 1, 0.1, 0.01, 0.001mg/cm^2) should span doses expected in field exposure.	Minimum 2, concentrate and field dilution.
Dose site occluded/ non-occluded area	Non-occluded back and shoulder (free of hair) at least 10 cm^2.	Non-occluded back and shoulders (free of hair) at least 10 cm^2.
Formulation solid	Commercial formulation, aqueous suspension.	Commercial formulation, dry powder.
Samples residual chemical	Mass balance approach urine, faeces, expired air, terminal blood exposed skin: washings from the dosed site and from the protective device at time of killing.	Mass balance approach urine, faeces, expired air, terminal blood exposed skin: washings from the dosed site and from the protective device at time of killing.
Exposure times	0.5, 1, 2, 4, 10, 24h.	8h.
Washing of application site	0.5, 1, 2, 4, 10, 24h.	8h.
Sample intervals	0.5, 1, 2, 4, 10, 24h.	8, 24 and 72h.
Results	Determination of the quantity and % absorbed by (a) addition of urine, faeces, blood and carcass data (b) subtracting skin wash and skin data from the dose applied.	Determination of the quantity and % absorbed; express results as unit weight of chemical absorbed per cm^2.
Additional studies	Where significant residues are found in the skin and no absorption is apparent after 24 h, longer time intervals may be required to establish the availability of this 'bound' material for absorption.	

* proposed protocol design.

TABLE 3 *IN VIVO* VS *IN VITRO* COMPARISON OF PERCUTANEOUS ABSORPTION DATA - HUMAN DATA

Compound	Reference	*In Vivo*	*In Vitro*	Ratio[1]
Acetylsalicylic acid	Franz, 1975	21.8	40.5	1.9
Benzoic acid	Franz, 1975	42.6	44.9	1.1
Benzoic acid (petroleum vehicle)	Bronaugh and Franz, 1986	60.6	41.8	0.7
Caffeine	Franz, 1978	22.1	24.6	1.1
Caffeine (water gel vehicle)	Bronaugh and Franz, 1986	4.0	4.7	1.2
Chloramphenicol	Franz, 1975	2.0	2.9	1.5
Dinitrochlorobenzene	Franz, 1975	53.1	27.5	0.5
Dinitrochlorobenzene	Bronaugh and Maibach, 1985	53.1	32.5	0.6
Fluazifop-butyl	Ramsey *et al*, in press	3.4	2.8	0.8
Hippuric acid	Bronaugh and Franz, 1986	1.0	1.35	1.3
Isofenophos	Wester *et al*, 1992	3.6	2.5	0.7
Nicotinamide	Franz, 1975	11.1	28.8	2.6
Nicotinic acid	Bronaugh and Franz, 1986	0.32	2.3	7.2
Phenol	Franz, 1975	4.4	10.9	2.5
Salicylic acid	Franz, 1975	22.8	12.0	0.5
Testosterone (water gel vehicle)	Bronaugh and Franz, 1986	49.2	41.4	0.8
Thiourea	Bronaugh and Franz, 1986	3.7	4.6	1.2
Urea	Franz, 1975	6.0	11.1	1.9

[1] Ratio *in vitro* data divided by *in vivo* data. Value of 1 means absolute agreement, if value <1 then *in vitro* under predicts *in vivo*, if value >1 then *in vitro* overpredicts *in vivo*.

With animal skin, probably the most comprehensive study has been reported for rat skin (Scott *et al*, 1992) with eight different chemicals, applied at a 1,000-fold range of doses, in a variety of vehicles. This study confirmed that the *in vitro* technique can predict *in vivo* absorption through rat skin (only a part of the extensive data set are presented in Table 4). Published studies have reported that *in vivo* absorption through, for example, mouse and monkey skin can be predicted from *in vitro* studies (see Table 4).

4.2.2. The Excised Skin *in vitro* Technique

The fundamental parameters underlying release, penetration and permeation can be studied by *in vitro* techniques. This does not necessarily mean that the membranes used have to be skin specimens. Artificial membranes are also used for such studies of penetration processes, especially in the development of pharmaceuticals (Jamoulle *et al*, 1989). Fuller details on *in vitro* procedures are given in Appendix D.

To separate percutaneous absorption from subsequent distribution, biotransformation and excretion processes, diffusion cells using human or animal skin and artificial membranes have been developed. Two chambered devices, sandwiching the skin or an artificial membrane between donor and receptor cell, or one-chambered cells with the receptor fluid directly beneath the membrane are at present in use.

TABLE 4 *IN VIVO* VS *IN VITRO* COMPARISON OF PERCUTANEOUS ABSORPTION DATA - ANIMAL DATA

Species	Compound	Reference	*in vivo*	*in vitro*	Ratio[1]
Rat	Anthracene	Yang *et al*, 1987	18	24	1.3
	Benzyl acetate	Hotchkiss *et al*, 1990	2	4	2
	Cypermethrin	Scott and Ramsey, 1987	1	1.7-2.7	1.7-2.7
	ICI A	Scott *et al*, 1992	12	24	0.5
	ICI B	Scott *et al*, 1992	86	76	0.9
	ICI C	Scott *et al*, 1992	23	22	1.0
	ICI D	Scott *et al*, 1992	56	79	1.4
	ICI E	Scott *et al*, 1992	4	3	0.8
	ICI F	Scott *et al*, 1992	32	78	2.4
	ICI G	Scott *et al*, 1992	2	2	1
	ICI H	Scott *et al*, 1992	14	9	0.6
Mouse	Permethrim	Grissom *et al*, 1987	26	18	0.7
	DDT	Grissom *et al*, 1987	25	27	1.1
	2,4-D	Grissom *et al*, 1987	44	28	0.7
	Fenvalerate	Grissom *et al*, 1987	19	20	1.0
Monkey	4-amino-2-nitrophenol	Bronaugh and Maibach, 1985	64	48	0.8
	2,4-dinitrochlorobenzene	Bronaugh and Maibach, 1985	52	48	0.9
	p-Nitroaniline	Bronaugh and Maibach, 1985	76	62	0.8
	Nitrobenzene	Bronaugh and Maibach, 1985	4.2	6.2	1.5

[1] Ratio, *in vitro* data divided by *in vivo* data. Value of 1 means absolute agreement, if value <1 then *in vitro* underpredicts *in vivo*, if value >1 then *in vitro* overpredicts *in vivo*.

All devices must be temperature controlled and can be run as static, stirred or as flow-through cells, the latter offering the advantage of automatic sample collection (cf. Appendix D). The test chemical either radiolabelled or non-radiolabelled, is applied in excess to the donor cell ("infinite dose" technique) or in thin films or layers in a vehicle to the surface of the skin ("finite dose" technique). The time course of appearance of the substance in the receptor cell is followed by a suitable radiometric or specific analytical method and the data evaluated as described in Section 5.

Human skin is not readily available to many investigators and this is likely to be the main obstacle to running reliable *in vitro* studies which predict human *in vivo* absorption. Depending on the aim of the study, animal skin can be used in many cases, especially for hazard assessment of industrial chemicals. Use of animal skins in *in vitro* studies can result in an overestimation of human systemic exposure (cf. Section 6). When the hazard assessment (the comparison of these exposure data with toxicological data from long-term toxicity studies) demonstrates a high enough safety factor, further *in vitro* or *in vivo* studies might not be necessary. Where the safety factor is not high enough a further study, e.g. with pig skin or with human skin *in vitro* or *in vivo* may be necessary.

There are some advantages in assessing percutaneous absorption by *in vitro* experiments in comparison with *in vivo* studies in that:

- conditions can be more easily controlled;
- normally, they are easier to perform;
- use of excised human skin is possible with radiolabelled or highly toxic chemicals;
- large numbers of experiments can be run simultaneously using skin from the same donor;
- reduced number of test animals;
- provides quantitative species comparison;
- special parameters (e.g. release of the chemical from different vehicles, influence of temperature, moisture, pretreatment on penetration) can be studied more readily and
- the amount of penetrating substance can be measured directly beneath the skin and without dilution in tissue fluids or organs. As a consequence studies with unlabelled substances are more feasible.

The disadvantages of *in vitro* methods are:

- the receptor solution may influence transfer of substance through the membrane (with very lipophilic substances it may be difficult to find a suitable receptor solution);
- blood vessels are beneath the epidermis and with excised skin the dermis (if whole skin samples are used) can act as an additional barrier, especially for lipophilic compounds;
- the excised skin membrane can only be maintained for short periods, this may influence any metabolic activity and destructive processes which influence absorption;
- *in vivo* conditions cannot be fully simulated *in vitro*, e.g. there is no shedding of *stratum corneum*;
- the penetration down hair and sweat ducts may be different *in vitro* than compared with *in vivo*;
- supplies of human tissue are limited and
- the methods are not fully accepted by regulatory authorities.

The technical limitations and pitfalls of the *in vitro* techniques can be minimised so that they can predict *in vivo* data. Thus with lipophilic chemicals especially, use of dermatomed, split thickness skin or epidermal membranes in combination with a suitable receptor solution can be highly recommended as a means of assessing percutaneous absorption if carefully evaluated in preliminary studies.

Recently, cultured skin preparations have become commercially available, for example the TESTSKIN® system (Organogenesis Inc., Cambridge, Mass.) which is based on differentiated cultured human keratinocytes and fibroblasts. This system is available in two versions, the Living Skin Equivalent (LSE™) and the Living Dermal Equivalent (LDE™). The living skin equivalent is morphologically similar to human skin and it is composed of two layers, a LSE epidermis with a cuboidal basal cell layer, stratified suprabasal cells and a differentiated *stratum corneum* similar to that found in human skin and

a thicker layer of human dermal fibroblasts in a collagen matrix. A similar system SKIN® is supplied by MARROW-TECH, La Jolla, California. Skin appendages, such as, hair follicles, sweat glands, sebaceous glands and the vasculature are lacking. These systems could be valuable and rapid tools for assessing percutaneous absorption and skin metabolism of chemicals, if thoroughly validated. First results indicate that cultured skin preparations possess drug metabolising enzyme activities but may be more permeable than human skin (Ponec, 1991).

Artificial membranes have also been used. A list of those used in diffusion cells is given in Table 5.

Table 5 Artificial Membranes used in Permeation Studies from Behl *et al*, 1990

Silastic® (polydimethylsiloxane)
Cellulose acetate membrane
Polyurethane membrane
Supor® (modified polysulfone)
Zeolite (aluminosilicates)
Multimembrane system (cellulose acetate: Silastic® cellulose acetate)
Diaflo-ultrafiltration membrane
Miscellaneous: collagen, egg shell
Liquid membranes (organic liquids, e.g. isopropyl myristate soaked on filter membranes)

Other types of membranes like the rotating diffusion cell (Ridout *et al*, 1990) or a special three compartment apparatus to study the rate of permeation across organic liquid layers have been described (Behl *et al*, 1990). The most extensively studied synthetic membranes, "Silastic®" and polyurethane membranes are simple homogeneous monolayer systems and cannot mimic the complex multilayered and heterogeneous barrier system of the skin. Artificial membranes can be used for the study of the physico-chemical aspects of the permeation process and may be used as a control to ascertain batch to batch uniformity of transdermal and topical drug formulations (Behl *et al*, 1990).

Assessment of the above literature indicates that cultured and artificial membranes are at present not a reliable alternative to skin for prediction of percutaneous absorption through human skin. Membrane models based on cultured human skin cell seem promising for the future but need extensive validation.

The available technical evidence indicates that data generated from *in vitro* percutaneous absorption studies performed and evaluated according to the guidelines described in Appendix D should be accepted by regulatory authorities.

4.3. OTHER MODELS

4.3.1. Stripping Method for Measuring Percutaneous absorption *in vivo.*

Chemicals usually have a low diffusion coefficient (10^{-10} - $10^{-12}cm^2/sec$) through the *stratum corneum*. Therefore, *in vivo* or *in vitro* skin penetration studies are usually performed over a long time period. Dupuis *et al* (1984), using the stripping method, found a linear relationship between the amount of chemical present in the *stratum corneum* at the end of application (30 min) and the total amount which permeated in 4 days. They suggest that the amount of chemical present in the *stratum corneum* at the end of 30min may be used to predict total absorption of the chemical (Rougier *et al*, 1989). Prediction of the steady-state rate of penetration is not possible with this method.

Tojo and Lee (1989) demonstrated that both the diffusion and the partition coefficient in the *stratum corneum* can be determined simply and quickly *in vivo* by measuring the amounts of chemical found in the *stratum corneum* after tape stripping at two short time intervals (10 and 30 min) after application. From the amount of chemical in the *stratum corneum* the steady-state rate of penetration can be calculated. These investigations were performed with the skin of hairless mice and validated with only two substances.

Another method using tape stripping was published by Wingen *et al* (1983). They applied dyes on the forearm of human volunteers. At certain time intervals tape stripping of the application area was performed and the amount of the dyes on each strip was measured photometrically. They were able to calculate the amount of dye which disappeared from the surface and therefore, the rate of absorption.

These procedures must be regarded as still in the development stage. Further studies are necessary to extend the stripping models to human skin and to broaden the data base. If these methods are validated with a broad range of chemicals they might be of value in predicting percutaneous absorption in man.

4.3.2. Specialised Models.

Several specialised skin preparations have been used to study absorption under conditions where the blood circulation system is functional and the skin is apparently physiologically normal. Such conditions are of particular importance when metabolism in skin or receptors is being investigated.

Porcine skin flap models (Monteiro-Riviere, 1990) have been shown to give good results but require highly specialised surgical facilities.

The athymic nude mouse readily accepts skin grafts from other species including man. The nude rat can also be used to support human skin grafts (Klain and Black, 1990). These grafts usually maintain morphological and functional properties, including normal blood circulation, and are metabolically active making them useful for answering specialised mechanistic questions which are not easily addressed by other techniques (Pershing and Krueger, 1989).

The rabbit ear may be a useful model to study skin metabolism by using perfusion techniques after dissection and cannulation of the main artery after the animal is killed.

These techniques are not suitable for routine percutaneous absorption measurements since they are labour intensive and require specialised facilities (Shaw *et al*, 1991).

4.3.3. Quantitative Structure-Activity Relationships (QSAR).

For the development of QSAR's it is essential to understand the physical processes involved in transport of a compound across the skin, many of which had been identified by early workers (Tregear, 1966). QSAR's relate percutaneous absorption data to chemical structure which can assist in building up a physiologically realistic model of skin transport (Hadgraft, 1990; Leahy, 1990). These processes are not yet fully understood, but justifiable simplifications and approximations can be made (Hadgraft, 1990).

It is believed that both hydrophilic and hydrophobic regions of the skin influence the skin absorption of a substance (Wester and Maibach, 1986). Partitioning and diffusion are considered to be the two key physical processes pertinent to dermal permeation (Guy and Hadgraft, 1989). An important physico-chemical constant of a compound, therefore, is the lipid-water partition coefficient. Attempts have been made to correlate the partition coefficient and the degree of skin absorption.

The best known and widely measured partition coefficient is that between n-octanol and water. Other systems, for instance n-heptane/water, lipid bilayer/water or *stratum corneum*/water have also been used (Flynn and Stewart, 1989). Surber *et al* (1990) were able to determine the partitioning of compounds between human *stratum corneum*/water and isopropyl myristate/water. The results from the solvent system were predictive of the *stratum corneum*/water data.

Correlations of partition coefficients with *in vivo* percutaneous absorption data have been made for a wide range of drugs (Bartek and LaBudde, 1975) including steroids (Anjo *et al*, 1980). The data show that the partition coefficient is not quantitatively predictive for percutaneous absorption. This is exemplified by the data published by Bartek and La Budde (1975) (cf. Table 6). All substances with a higher partition coefficient (more soluble in heptane than in the used buffer) showed at least a moderate *in vivo* absorption rate through the skin of man, pig or rabbit. Substances with a low partition coefficient were not always poorly absorbed cutaneously. Determination of the partition coefficient does

allow a distinction to be made between compounds with a potential for high and low absorption (Wester and Maibach, 1986; Guy and Hadgraft, 1989). Predictions have been found to be closer within related chemical series (Leahy, 1990).

TABLE 6 COMPARISON OF PERCUTANEOUS ABSORPTION IN DIFFERENT SPECIES WITH PARTITION COEFFICIENT (Bartek and La Budde, 1975)

Substance	% of the applied amount absorbed			Partition coefficient Heptane/buffer[1]
	Man	Pig	Rabbit	
Butter yellow	21.6	41.9	100	101
Haloprogin	11.0	19.7	>100	45.4
Malathion	8.2	15.5	64.6	33.2
Lindane	9.3	37.6	51.2	32.9
DDT	10.4	43.4	46.3	28.5
Parathion	9.7	14.5	97.5	14.4
Testosterone	13.2	29.4	69.6	1.81
Caffeine	47.6	32.4	69.2	0.005
Cortisone	3.38	4.06	30.3	0.002
N-Acetylcysteine	2.43	6.00[2]	1.98	0.0004

1 expressed as concentration of substance in heptane divided by concentration of test compound in buffer.
2 limit of sensitivity

The QSAR approach cannot take into account the influence of any metabolism in the skin. In investigations performed with a radiolabelled compound it is often not possible to distinguish between the parent compound and its metabolites (Wester and Maibach, 1985a,b). For that reason the best correlations are obtained for those chemicals for which no metabolism occurs.

Kasting *et al* (1987) analysed the permeability data on 35 drugs of a diverse range. In addition to the log octanol/water partition coefficient, other parameters were included in their model system such as molecular volume, melting point and solubility in propylene glycol, n-octanol and isopropyl myristate. There was no simple mathematical relationship between the permeability and any individual parameter but there was an indication that both n-octanol/water partition coefficient and molecular volume correlated well with absorption. Other parameters which influence the diffusion of lipophilic molecules were melting point and molecular weight.

Anderson and Raykar (1989) examined the penetration of a series of lipophilic cresol derivatives and 21-esters of hydrocortisone through human *stratum corneum.* They related the penetration to a range of partition coefficients including octanol/water, heptane/water, *stratum corneum*/water and delipidised *stratum corneum*/water. Additional parameters considered were the nature of functional groups $-CH_2-$, $-CONH_2$, $-CON(CH_3)_2$, $-COOCH_3$, -COOH and -OH, on the free energies of transfer from water to lipid phases. These analyses showed the importance of the more highly polar groups in reducing diffusion

of molecules through skin. Permeability coefficients determined in *in vitro* investigations (Blank *et al*, 1967; Scheuplein *et al*, 1969; Roberts *et al*, 1975; Roberts *et al*, 1977; Kasting *et al*, 1987; Anderson and Raykar, 1989) have also been used in QSAR analyses.

It should be noted that the present QSAR approach does not take into account the interaction of chemicals with the epidermal membranes, e.g. such as may cause corrosion, which could affect the skin permeability. With the advent of readily available computing systems, more elaborate mathematical modelling will be developed which may allow a more reliable prediction of absorption through the skin.

The available data are derived from a range of protocols and are too inconsistent to permit a generally useful QSAR approach. Further studies and improvement of the models would allow the establishment of a quantitative data base which could be rigorously analysed to relate physico-chemical parameters and skin permeability. This could be useful for screening of new chemicals according to their percutaneous absorption rate potential.

SECTION 5. PRESENTATION OF RESULTS

5.1. INTRODUCTION

A comparison of data from *in vivo* and/or of *in vitro* percutaneous absorption studies is often difficult or impossible as basic details of the experimental design are not reported. To improve the current situation, data should include the information as indicated subsequently.

5.1.1. Test substance

Full details of physico-chemical properties should be reported of the test substance including site of radiolabel, specific radioactivity, radiochemical purity, pK_A-value, melting point, solubility in water and n-octanol/buffer (pH 7) partition coefficient.

5.1.2. Test Preparation

The test preparation should be characterised with respect to composition, pH and concentration of the substance of interest. The physico-chemical state of the penetrant in the vehicle (e.g. whether in solution or suspension) should be reported. Ideally, the stability of the test substance in the vehicle at the time of application should be proven by a suitable analytical procedure.

5.1.3. Application of the test substance

The anatomical site to which the preparation is applied should be reported along with information on pre-treatment (electrical clipping, shaving, depilation, cleansing procedures etc.) and the size of treated skin area. The test preparation should be applied as evenly as possible. The volume or amount of formulation applied per cm^2 of skin must be defined and the exposure time, i.e. the time from application until the preparation is rinsed from the skin. Experience has shown that *in vivo* the maximum amount of a compound which can be retained on human skin is around $5 mg/cm^2$ of a solid deposit or $5 \mu l/cm^2$ of a liquid. An increase of the dermal area dose over 5mg formulation per cm^2 did not further increase percutaneous fluxes (Walker *et al*, 1991).

5.2. *IN VIVO* STUDIES

5.2.1 Percutaneous Absorption Determined from Excreta Measurements

This widely practiced toxicokinetic experiment uses a single dermal application of radiolabelled test chemical followed by a quantitative analysis of the excreta. Small animals like mice, rats and guinea pigs are killed after predefined dermal exposure periods and the radiolabelled substances in the

carcass (exclusive of the application site) is determined. Percutaneous absorption is calculated from the sum of the cumulated radioactivities in the excreta and the residue found in the carcass.

In larger animals like the dog, monkey and pig and also man, percutaneous absorption is assessed only from the cumulated excretion of radiolabelled substances in urine and faeces. In these species excreta have to be sampled far beyond the exposure period, possibly until the detection limit for the radiolabelled substances in excreta has been reached (Marco *et al*, 1985; Van Lier, 1985).

The amount of chemical present in any protective device and in skin rinsings, is considered not to have penetrated the skin. The amount of radiolabel in the skin is usually determined in smaller animals by excision of the application site (Farber and Zendzian, 1990).

The amount present in the skin can be determined by adhesive tape stripping (10 to 20 times) of the application site before skin excision. Subsequent extraction of radiolabel from the tapes, allows the portion in the *stratum corneum* to be separated from that in the deeper layers of skin. Adhesive tape stripping can also be used with larger animals and man where skin excision is not performed. A chemical found in the deeper layers of living skin has already passed through the *stratum corneum* barrier and is therefore considered to have been absorbed. The portion in the *stratum corneum* should be regarded as having a potential to be absorbed at a later time.

In "finite dose" experiments, in which a defined area of the skin is exposed to a chemical in thin films or layers for a defined period of time, percutaneous absorption is most simply expressed as percentage of the dose applied. Knowing the treatment area and the exposure time a mean substance flux (expressed in mass units per cm^2 per hour) can be calculated. Since in these experiments the substance flux through the skin is usually time dependent, due to the decrease of concentration of the test chemical on the skin with time, only average fluxes may be calculated.

In "infinite dose" experiments (e.g. where the hand is immersed in a solvent or when large volumes of a solvent or formulation are placed in a chamber on the skin) a constant percutaneous flux through the skin may be expected after a lag-time. This constant flux leads to steady state plasma levels and to a constant excretion rate of substance or radiolabel in excreta (Ravis, 1990). In this case expression of the results as percentage of the applied dose usually gives very low values and is not the most useful figure. Expression as the percutaneous flux (amount/area/time) is a better alternative.

5.2.2 Percutaneous Absorption Determined from Plasma or Urine Measurements

This type of experiment is usually performed during the development of transdermally or topically applied drugs. It does not require the application of radiolabelled substance but a sensitive and specific method for determination of the parent drug in the plasma or urine (assuming urinary excretion) must be available. In addition to the dermal application an intravenous injection of a defined dose, ideally

done in the same subjects, has to be performed. Drug plasma and/or urinary levels after both administrations are followed until limits of detection are reached. The extent of percutaneous absorption F (= absolute bioavailability) of the compound can be calculated by comparing areas under the drug plasma concentration curves (AUC^{∞}) or by comparing the total amount of parent drug excreted with the urine (U_e^{∞}) taking into account the different intravenous and dermal doses ($D_{topical}$).

$$F = \frac{AUC^{\infty}_{topical} \cdot D_{iv}}{AUC^{\infty}_{iv} \cdot D_{topical}} \cdot 100 \qquad (1)$$

$$F = \frac{U^{\infty}_{e\ topical} \cdot D_{iv}}{U^{\infty}_{e\ iv} \cdot D_{topical}} \cdot 100 \qquad (2)$$

The extent of percutaneous absorption can be expressed as a ratio < 1 or as percentage of the topical dose applied as given in equations (1) and (2).

If a radiolabelled substance is applied and the metabolites of the test compound are independent of dose and route of administration, percutaneous absorption can be calculated from the cumulated excretion of radioactivity in urine using equation (2).

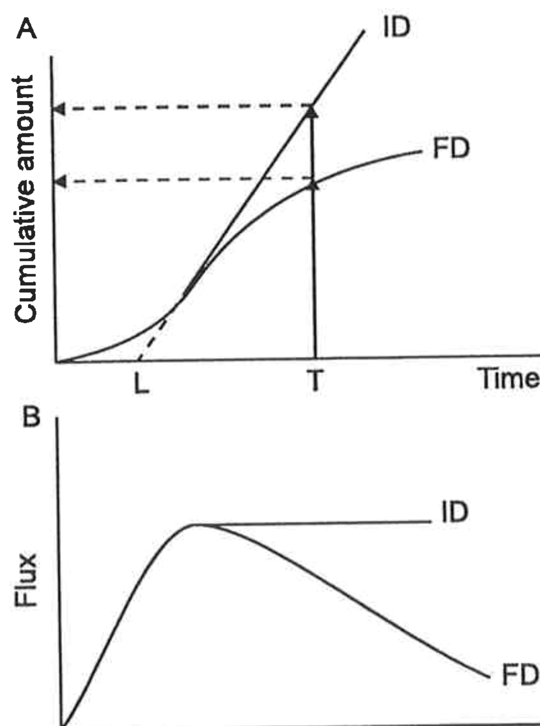
5.3. *IN VITRO* STUDIES

In vitro percutaneous absorption studies use excised i.e. *ex vivo* skin. In these experiments using skin diffusion cells, the time course of the appearance of either radiolabelled or parent chemical in the receptor fluid is monitored. A typical plot of the cumulated amount of a test compound which has permeated a defined skin area A is shown as a function of the time in Figure 2 for an "infinite dose" and a "finite dose" experiment. Also in Figure 2, the substance flux, i.e. the slope (1st derivative) of the cumulated amount of a test compound divided by the skin area, is presented as a function of the time.

In case of an "infinite dose" experiment (Figure 2 curve ID) the substance flux through the skin (mass units per cm^2 per h) increases after a lag time until a constant steady state flux is reached. The steady state flux can be calculated by dividing the slope of the regression line through the linear part of curve ID of Figure 2 by the area of treated skin. The intercept of the straight line on the abscissa represents the lag time L.

In case of a "finite dose" experiment (Figure 2 curve FD) the substance flux through the skin increases after a lag time, passes a maximum and then decreases due to depletion of the chemical in the formulation on the skin surface. Cumulated amounts of substance penetrating a unit area of skin within

Figure 2 **Cumulative Amount of Penetrant in the Receptor Cell (A) and Percutaneous Flux (B) as a Function of Exposure Time for a Finite (FD) and an Infinite Dose (ID) Experiment (after application of the penetrant to the skin in the same vehicle).**



a given exposure period can be obtained directly from the curves in Figure 2 by dividing the respective value of the ordinate by the skin area used in the *in vitro* experiment. The cumulated amount may also be expressed in percentages of the dose applied but this is meaningful only for the "finite dose" situation.

More details are given in Appendix D.

5.4. PRESENTATION OF RESULTS

Preceding sections have described the types of data generated in *in vivo* or *in vitro* percutaneous absorption studies. Published data can be difficult to interpret and compare due to differences in the study protocols used. Presentation of the experimental details, as recommended in this document, will facilitate inter-study and inter-compound comparisons.

Absorption data are commonly expressed as "% applied dose absorbed" per unit of time. Provided the information recommended in this document is available this is readily calculated, as illustrated in the

following three examples. These calculations illustrate some of the problems inherent in presenting the results of percutaneous absorption studies in this manner.

Example 1. Compound A

Dermal area dose	0.5mg/cm^2
Exposure period	10hr
Systemic exposure (Determined amount absorbed)	10µg

% dose absorbed $\frac{10\mu g}{500\mu g} \times 100 = 2.0\%$ in 10hr

% dose absorbed $\frac{2\%}{10 \text{ hours}} = 0.2\%$ per hr

(This type of data must be considered of limited value: see later)

Example 2. Compound A

Dermal area dose	5mg/cm^2
Exposure period	10hr
Systemic exposure (Determined amount absorbed)	50µg

% dose absorbed $\frac{50\mu g}{5{,}000\mu g} \times 100 = 1.0\%$ in 10hr

% dose absorbed $\frac{1\%}{10 \text{ hours}} = 0.1\%$ per hr

Example 3. Compound A

Dermal area dose	50mg/cm^2
Exposure period	5hr
Systemic exposure (Determined amount absorbed)	40µg

% dose absorbed $\frac{40\mu g}{5{,}000\mu g} \times 100 = 0.8\%$ in 5hr

% dose absorbed $\frac{0.8\%}{5 \text{ hours}} = 0.16\%$ per hr

These data are of limited value unless the test compound is absorbed linearly with both dermal dose and with time which occurs rarely. It must, therefore, be recognised that any result presented in this manner is relevant only to a particular dose and a particular time: extrapolations to other situations are difficult. In example 1, 2.0% of the compound was absorbed over 10 hours, whereas in example 2 only 1.0% was absorbed. This might be misinterpreted as indicating that the compound is absorbed in larger amounts from example 1 (0.5mg dermal dose) than example 2 (5mg dermal dose), and this, of course, is wrong. Only if dermal doses and exposure times are standardised between experiments are "% dose absorbed data" useful for comparisons.

Unfortunately, the same problems are associated with the presentation of results in other ways. Examples 1 and 2 show that the total amounts absorbed at 10hr are influenced by the dermal dose and these amounts are in turn a function of the exposure period. Conversion of these data into absorption rates can also be misleading unless the rate is constant during the exposure period (and, of course, any real rate will be influenced by the dermal dose). This is illustrated in Figure 2 where for both infinite and "finite doses" the rates of absorption change over time. In specifying precisely which rate(s) should be calculated and consideration should be given to the ultimate use of the data.

If rates are calculated, the time period over which they relate must be given. If rates change over an exposure period then, perhaps, more than one rate must be calculated (which may be difficult in *in vivo* experiments) or only an approximate rate calculation will be possible.

Consideration of the above indicates that the initial data are probably best presented in terms of "amount" at a specific time; subsequent calculations can be done as necessary. The absolute definition of the actual time should, again, be made with regard to the use of the data. For most general purposes absorption during a typical work period of 8 hours and during 24 hours should be determined. These time periods would be relevant to accidental dermal exposure and those occurring from "leave-on" products. Rates of absorption can then be calculated over other time periods of interest.

The above is intended to illustrate the problems in the presentation of data. Without linear relationships between absorption, dose and time, great care must be taken in the way experimental data are expressed. Standardisation of protocols will improve interstudy comparisons but, generally, comparisons of data sets must be done cautiously.

SECTION 6. RELEVANCE TO MAN OF ANIMAL *IN VIVO* OR ANIMAL AND HUMAN *IN VITRO* DATA

6.1. INTRODUCTION

The ultimate objective of dermal absorption experiments is to identify a potential hazard to the human population. This section describes conclusions derived from the comparative data reported in the literature on the ability of studies other than human volunteer studies to predict human *in vivo* absorption.

6.2. ANIMAL *IN VIVO* DATA

A summary of studies reported in the literature which have compared dermal absorption of chemicals in different animal species and man is given in Table 7.

Direct comparison between experiments and species is not always possible due to the differences in methods used. These data indicate that the permeability of pig and monkey skin is generally lower than other animal species and more like that of man whereas mouse, rabbit, rat and guinea pig skin is more permeable. One exception seems to be with N-acetylcysteine to which pig skin appears to be more permeable than human skin, while rabbit skin is most like man (Bartek *et al*, 1972).

TABLE 7 THE PERMEABILITY OF ANIMAL AND HUMAN SKIN TO CHEMICALS IN VIVO (all values presented are % applied dose absorbed unless otherwise stated, numbers of individuals tested are presented in parenthesis).

TABLE 7.i

Compound	Rat	Monkey	Comments	Reference
Lindane	31(5)	forepaw 54(4) forehead 34(7)		Moody and Ritter, 1989
N,N-Diethyl-m-toluamide	36(8)	forearm 18(8) forepaw 68(8) forehead 33(8) forepaw 27(8) forearm 14(8)		Moody *et al*, 1989
Fenitrothion	84(5)	forehead 49(8) forearm 21(8)		Moody and Franklin, 1987
Amino carb.	86(5)	forehead 74(8) forearm 37(8)		

TABLE 7.ii

Compound	Pig	Hairless Dog	Hairless Mouse	Skin Graft H/M[1]	Skin Graft P/M[2]	Comments	Reference
Testosterone	5(3)	12(3)	73(3)	43(3)	41(3)	Chemicals applied at 4µg/cm^2	Reifenrath *et al*, 1984b
Caffeine	20(3)	21(3)	70(3)	44(3)	34(3)		
Lindane	6(3)	8(3)	55(3)	21(3)	24(3)		
Parathion	18(3)	20(3)	26(3)	71(3)	27(3)		
Malation	4(3)	12(3)	77(3)	31(3)	24(3)		
Benzoic acid	12(3)	22(3)	54(3)	40(3)	42(3)		
N,N-Diethyl-m-toluamide	7(3)	12(3)	55(3)	26(3)	29(3)		
Progesterone	7(3)	27(3)	81(3)	35(3)	48(3)		
Fluocinolone acetonide	6(3)	5(3)	49(3)	28(3)	21(3)		

1 H/M human skin grafted to mouse.
2 P/M pig skin grafted to mouse.

TABLE 7.iii

Compound	Rat	Rabbit	Pig	Human	Comments	Reference
Halopogin	100(4)	113(3)	20(2)	11(6)	Chemicals applied at 4µg/cm^2/24h.	Bartek *et al*, 1972
N-Acetylcysteine	4(4)	2(3)	6(2)	2(6)		
Testosterone	47(4)	70(3)	29(2)	13(17)[1]		
Cortisone	25(4)	30(3)	4(2)	3(7)[1]		
Caffeine	53(4)	69(3)	32(2)	48(12)[1]		
Butter yellow (p-dimethyl-aminoazo-benzene	48(4)	100(3)	42(2)	22(4)[1]		

1 Human data taken from Feldman and Maibach (1969, 1970).

TABLE 7.iv

Compound	Rabbit	Pig	Monkey	Human	Comments	Reference
DDT	46(3)	43(2)	2(4)[1]	10(6)[2]	Chemicals applied at 4µg/cm^2 /24h.	Bartek and La Budde, 1975
Lindane	51(3)	38(2)	16(2)	9(6)[2]		
Parathion	98(3)	15(2)	30(2)	10(6)[2]		
Malathion	65(4)	16(2)	19(2)	8(6)[2]		

1 Not corrected for parenteral administration
2 Human data taken from Feldman and Maibach (1974).

TABLE 7.v

Compound	Monkey	Human	Comments	Reference
Testosterone Hydrocortisone Benzoic acid,	18(8) 3(3) 59(3)	13(17)[1] 2(15)[1] 42(6)[1]	Values for benzoic acid have not been corrected for incomplete uninary excretion. Benzoic acid applied in MeOH others in acetone at 4µg/cm².	Wester and Maibach, 1975
Testosterone Hydrocortisone Benzoic acid	2(3) 2(3) 17(3)	3(6) 1(5) 14(7)	400µg/cm² dose level. 40µg/cm²dose level. 2000µg/cm² dose level Values not corrected for incomplete urinary excretion. Benzoic acid applied in MeOH others in acetone.	Wester and Maibach, 1976
Nitrobenzene 2,4-Dinitrochloro-benzene	4(6) 53(4)	2(4) 53(4)		Wester and Maibach, 1989b

1 Human data from Feldman and Maibach (1969,1970).

6.3. ANIMAL AND HUMAN *IN VITRO* DATA

The skins used commonly for *in vitro* percutaneous absorption studies are from rats, mice, rabbits, guinea pigs, mini-pigs and pigs. For a number of compounds permeability values comparable to human skin were found *in vitro* with rat skin (Bronaugh *et al*, 1982b; Scott *et al*, 1986a, b) (Table 8).

TABLE 8 THE PERMEABILITY OF ANIMAL SKIN RELATIVE TO HUMAN SKIN IN VITRO (values presented are % applied dose absorbed)

TABLE 8.1

Compound	Human	Pig	Rat	Guinea pig	Rabbit	Reference
Ethylene bromide	1	0.8[1]	2.3[1]	1.5[1]		Tregear (1966)
Paraoxon	1	1.4[1]	3.3[1]	3.0[1]		
Thioglycolic acid	1	3.3[1]	3.0[1]	2.3[1]		
Water	1	1.4[1]	3.0[1]	1.0[1]	3.3	

1 These values are ratios. Human skin was assigned the value 1.0 (from Bronaugh and Maibach, 1987).

TABLE 8.2

Compound	Human	Hairless mouse	Reference
Betamethasone-17-valerate	0.7	0.8	Stoughton (1975)[1]
Betamethasone	0.7	0.9	
Thiabendazol	9.4	14.6	
Tolnaftate	0.9	1.4	
Hydrocortisone	1.3	1.9	
5-Fluorouracil	14.2	16.1	

[1] Values are the mean % of the applied dose.

TABLE 8.3

Compound	Human	Pig	Rat	Hairless mouse	Mouse	Reference
Benzoic Acid[1]	8.1	2.0	5.0	17.1	17.6	Bronaugh *et al* (1982b)
Acetylsalicylic Acid[2]	4.2	5.5	4.5	21	37	
Urea[2]	0.5	0.6	1.8	0.5	2.3	

[1] Values are expressed as the mean permeability coefficient [cm hr-1 x 10^{-5}]. Rodent skin: whole skin; pig skin: sliced skin; human skin: epidermal membrane

[2] Values are expressed as the mean permeability coefficient [cm hr-1 x 10^{-5}]. Rodent skin: whole skin; pig skin: sliced skin; human skin: epidermal membrane
Values were calculated from the fig. 1 of the paper of Bronaugh *et al,* (1982b).

TABLE 8.4

Compound	Human	Rat	Mouse	Reference
1-Chloro-2,4-dinitro-benzene	286.1 ± 52.5	1161 ± 228	1737 ± 257	Scott *et al,* (1986b)[1]

[1] Values are expressed as the mean absorption rate [μg cm-2 h-1] ± standard error. Epidermal membranes.

TABLE 8.5

Compound	Human	Pig	Rabbit
DDT	0.5	0.5	Hawkins and Reifenrath (1986)[1]
Benzopyrene	1.0	0.7	
Fluocinolone acetonide	2.2	1.0	
Progesterone	2.3	5.3	
Lindane	5.3	6.3	
Testosterone	4.0	8.9	
Parathione	4.2	12	
Diisopropylfluoro-phosponate	18	14	
Malathion	12	21	
Benzoic Acid	29	17	
Caffeine	29	28	

1 Values are derived from Fig. 2 of the paper of Hawkins and Reifenrath (1986). Values are expressed as the mean % of the applied dose. Split thickness skin.

TABLE 8.6

Compound	Human	Pig	Monkey	Rat	Guinea pig	Hairless Mouse	Mouse	Reference
Water	93 ± 14	389 ± 151[2]	103 ± 11	442 ± 32	351 ± 52	144 ± 17	253 ± 45	Scott *et al* (1986a)[1]
Paraquat	1 ± 0.2	5 ± 2[2]	27 ± 4	196 ± 10	1066 ± 135	97 ± 16	80 ± 17	

1 Values are expressed as the mean permeability coefficient [cm hr-1 x 10^{-5}] ± standard error. Whole skin.

2 Values from Bhatti *et al* (1988)

TABLE 8.7

Compound	Human	Pig	Rat	Reference
n-Butoxyethanol	7	9	11	Bartnik *et al* (1987)[1]

1 Values are expressed as the mean % of the applied dose. Whole skin.

TABLE 8.8

Compound	Human	Rat	Reference
Dimethylphthalate	3.32 ± 0.54	34.5 ± 3.51	Scott *et al* (1987)[1]
Diethylphthalate	1.14 ± 0.10	37.0 ± 8.3	
Dibutylphthalate	0.23 ± 0.06	8.95 ± 0.09	
Di-(2-ethylhexyl)-phthalate	0.57 ± 0.12	2.28 ± 0.23	

1 Values are expressed as the mean permeability coefficient [cm hr-1 x 10^{-5}] ± standard error. Epidermal membranes.

TABLE 8.9

Compound	Human	Monkey	Reference
p-Nitroaniline	48.0 ± 11.0	62.2 ± 6.9	Bronaugh (1989a)[1]
4-Amino-2-nitrophenol	45.1 ± 8.0	48.2 ± 7.8	
2,4-Dinitrochlorobenzene	32.5 ± 8.7	48.4 ± 3.9	
2-Nitro-p-phenylenediamine	21.7 ± 2.6	29.6 ± 4.3	
Nitrobenzene	7.8 ± 1.2	6.2 ± 1.0	

1 Values are the mean % of the applied dose ± standard error. Whole skin.

TABLE 8.10

Compound	Human	Rat	Guinea pig	Mouse	Rabbit	Reference
Diquat dibromide	0.56 ± 0.56	2.32 ± 0.75	4.55 ± 0.34	3.33 ± 0.39	4.31 ± 0.41	Scott and Corrigan (1990)[1]

1 Values are expressed as the mean permeability coefficient [cm hr-1 x 10^{-4}] ± standard error. Whole skin

The skin of hairless mice has also been used in *in vitro* studies. Results similar to those with human skin have been reported for some compounds (Stoughton, 1975; Durrheim *et al*, 1980) but Bronough *et al*, (1982b) have shown that this may depend on the nature of the compound. Data from other investigations suggest that the skin of hairless mice is so thin that it can substantially overestimate some effects (Bond and Barry, 1988a,b). The hairless mouse skin was found to be > 1000 times more permeable to paraquat and 3.8 times to water than for human skin in an *in vitro* study with whole skin (Scott *et al*, 1986a). The percutaneous absorption rate of some dyes measured with different animal skin was highest through skin of hairless mice (Bartnik and Zimmermann, 1988). In the study of Marzulli *et al*, (1969) it was found that hairy mouse skin was much more permeable than human skin.

The use of hairless guinea pig skin has been advocated recently (Bronaugh *et al*, 1989b; Behl *et al*, 1990) but it remains to be demonstrated further that it is useful for *in vitro* studies and for prediction of human absorption.

Pig skin has also been used in *in vitro* investigations (Hawkins and Reifenrath, 1984, 1986; Bracher *et al*, 1987; Noser *et al*, 1988).

A comparison of the absorption data for many of these studies, for different, mostly water-soluble chemicals, is summarised in Table 8. The same type of skin membrane could not be used for the interspecies comparison in all cases as epidermal membranes can only be prepared from a limited number of animal species and whole or split-thickness skin had to be used. Higher penetration rates were generally found with animal skin than with human skin.

From the limited data it is concluded that the skin of monkey and pig are the most promising for use as an *in vitro* model for human skin. This conclusion does not exclude the use of skin of other animal species for special investigations.

SECTION 7. EXPOSURE ASSESSMENT

7.1. INTRODUCTION

The surface of human skin is exposed to chemicals intentionally with pharmaceutical products, cosmetics, bathing lotions etc. or unintentionally with dust particles, contaminated water, household chemicals etc. or at the workplace with industrial chemicals such as solvents, agrochemicals etc. During the process of hazard assessment, estimation of the amount of chemical in contact with human skin, "dermal exposure", and as a consequence of the percutaneous absorption the "systemic exposure" have to be quantified or predicted. Details of the methods which may be used to quantify these exposures are presented in Appendix E.

7.2. ASSESSMENT OF DERMAL EXPOSURE

Total dermal exposure can be defined as the total amount of a chemical in contact with the skin surface for a specified exposure time. It can be calculated as a product of the dermal area dose in amount of chemical per cm^2 of skin and the exposed area of the skin in cm^2. In all cases the duration of the dermal exposure must be stated, because it influences the extent of the systemic exposure.

7.2.1 Exposure to Consumer Products

For products which are designed to contact the skin, such as cosmetics and cleansing agents, the estimation of skin exposure is relatively simple. It can be expressed as:

Dermal Exposure (mg/day) = amount per application (mg) x
frequency of application per day.

COLIPA listed average use of cosmetic products (cf. COLIPA, 1981 presented in Appendix B).

For "leave-on" products, such as skin creams, this dermal exposure estimation is accurate but for "rinse-off" products such as shampoos, soaps and hair dyes, the reduction of exposure by rinsing is not easy to assess. The amount of chemical remaining in reservoirs on or in the *stratum corneum* prior to rinsing is difficult to predict. A simple method for determining the magnitude of such reservoirs is by analysis of the skin rinsings after applications of known amounts of test material (Black and Howes, 1975; Howes, 1975; Howes and Black, 1983). The loss of material from the amount applied gives an estimate of the skin reservoir on or in the skin.

7.2.2 Worker Exposure to Industrial Chemicals

The amount of chemical potentially available for absorption through the skin can be estimated by trapping it before it contacts the skin, or by removing material that has contacted the skin before it is absorbed.

This is generally done using various types of pads (patches) or articles of clothing (e.g. disposable coveralls) to trap impinging residues, or by removing residues from the skin by swabbing or rinsing with a solvent (Durham and Wolfe, 1962; Davis, 1980; Lewis *et al*, 1980; Maddy *et al*, 1980; cf. Appendix E).

Dermal exposure may be calculated using the individual patch data. The amount of chemical per cm^2 of patch is multiplied by the total specific body area in question (Table 1). If dermal area doses vary in different body regions it is recommended that surface areas derived from Popendorf's anatomic model are used (Popendorf and Leffingwell, 1982); these are already adopted by the World Medical Association (WMA, 1982) for dermal exposure calculations. The individual exposures are summed up to give total dermal exposure in mg of the chemical. If the amount of material on the patch is not representative of the general exposure on the body part then the calculated dermal exposure might be inaccurate. If coveralls have been used to assess exposure, they may be cut into individual body regions, and the whole of the material analysed and the dermal exposure calculated.

In some cases it is reasonable to express total dermal exposure in terms of the mg/kg chemical handled. This value is then adjusted for the number of chemicals handled during a normal working day to arrive at an estimated daily dermal exposure value expressed in mg chemical/day (Franklin *et al*, 1986, 1989).

The development of a generic exposure data base for pesticides is one of the major projects of the National Agricultural Chemical Association (NACA, 1985; Honeycutt, 1986) on exposure assessment. The purpose is to provide equivalent mixer-loader-applicator exposure data to EPA, industry and the public in order to standardise exposure assessment (Honeycutt, 1986).

With the exception of the pesticides only few dermal exposure studies of occupational exposed workers are reported (Koizumi, 1991; Johanson and Boman, 1991).

7.3. ASSESSMENT OF SYSTEMIC EXPOSURE

Skin exposure and possible percutaneous absorption will make chemicals available systemically to the human body.

There are three different approaches to systemic exposure assessment. Where no information on the magnitude of percutaneous absorption is available, it might be assumed for safety reasons that the dermally deposited dose is completely absorbed (systemic exposure = dermal exposure). This overestimates systemic exposure.

In the case that *in vivo* or *in vitro* percutaneous absorption studies (Sections 4 and 5) have been performed, results can be used to convert dermal exposure values into systemic exposure values (systemic exposure < dermal exposure).

Systemic exposure can also be assessed by biological monitoring. This does not require information on the magnitude of the dermal exposure but requires knowledge of the metabolism and pharmacokinetics of the chemical.

When relating sub-chronic or chronic toxicity to human exposure data, it is recommended that systemic exposure is expressed in mg/kgbw/d assuming an average adult male weight of 60 kg.

7.3.1. Assessment of Systemic Exposure Using Percutaneous Absorption Measurements

Exposure to Consumer Products. Dermal exposure to leave-on consumer products is well defined and the systemic dose can be calculated by multiplying the dermal exposure level by the absorption factor (cf. Section 5). Care must be taken that this factor was obtained using relevant experimental conditions (appropriate exposure, vehicle, concentration, area dose, duration of exposure, cf. Section 3). If percutaneous substance fluxes have been calculated, systemic exposure can be calculated by multiplying the percutaneous flux with the exposure time and the area of skin covered by the product. In this case extrapolation to shorter exposure times is possible.

It may be appropriate when predicting systemic exposure to consider the relative permeability of the exposed body areas (cf. Table 1). Use of relative permeability indices for a prediction of total body exposures may be justified, but possibly only for chemicals with similar physico-chemical characteristics to those substances for which regional absorption data are available. Only limited data are available. It is probable that variations in body site permeability need to be considered only for those chemicals which are deliberately applied to particularly permeable areas, e.g. axilla and scalp.

Dermal exposure is less well defined for rinse-off products because of variations in consumer habits and product type and percutaneous absorption fractions must be determined under relevant experimental conditions. For assessment of systemic exposure, the same calculations can be used as for leave-on products.

Exposure to Industrial Chemicals. For the industrial situation, the same principles can be applied to estimate the maximum likely systemic exposure as described above (cf. Section 8) but the dermal

exposure in the workplace is usually poorly defined. Adverse local skin effects constitute almost 50% of reported case of occupational disease (ECETOC, 1990a,b). Workers are likely to have mixed exposures, e.g. to solvents, wash and coolant solutions, aromatic amines, nitro compounds, styrene etc. (Ahmed and Trieff, 1983; Bolt and Peter, 1984; Buchter and Peter, 1984; Wieczorek, 1985; Lewalter, 1990; Scott and Corrigan, 1990; Koizumi, 1991; Roberts *et al*, 1991). It can be difficult to differentiate whether effects result from inhalation and dermal exposure. Inhalation exposure often constitutes the major part of the total exposure but in some cases exposure via skin has accounted for more than 90% of the total exposure. Extended dermal exposure to solvents can induce toxic effects such as depression of the central nervous system, anemia and effects on bone marrow, liver and kidney damage (Scott and Corrigan, 1990; Long, 1991). Asphyxia was reported after accidental dermal overexposure to acrylonitrile (Lewalter, 1990)) and nephrotoxic effects after exposure to phenol (Foxall *et al*, 1991).

From human or animal studies with exposures up to 8 hours (a typical working day period) it has been estimated that dermal uptakes range from 0.1 to 10% of the total uptake for non-polar chemicals such as benzene, dibromomethane, halothene, hexane, mercury, styrene, perchloroethylene, toluene, 1,1,1-trichloroethane and xylene (Johanson and Boman, 1991). Such a generalised view is not valid for all such substances, for example with ethylene glycol mono methyl ether (EGME) for which the dermal uptake from vapours is appreciably higher than the respiratory uptake (Johanson and Boman, 1991).

In view of those uncertainties biological monitoring of exposure may be appropriate when workers are exposed to industrial chemicals which are absorbed percutaneously.

7.3.2. Assessment of Systemic Exposure using Biological Monitoring

Biological monitoring can be used to detect whether an individual has been exposed to and has absorbed a chemical (Zielhuis and Henderson, 1986; Lehnert, 1980; Leung and Paustenbach, 1988). This can be ascertained by measuring the parent chemical or characteristic metabolites in blood, urine, erythrocytes, lymphocytes, saliva, sweat, expired air, hair or adipose tissue. To quantitate systemic exposure, biotransformation and pharmacokinetic data are essential.

The occurrence of a chemical or its metabolites in tissues or excreta is evidence of dermal exposure only if other routes of exposure (inhalation and ingestion) have been excluded. Exposure assessment strategies based on biological monitoring must therefore be designed to take into account exposures through skin and by other routes and to integrate the consequences of intermittent as well as continuous exposures. They can, however, provide evidence of human dermal exposure to multiple chemicals (Schulte, 1988; Cullen, 1989; Wogan, 1989; DeFlora, 1990).

Biological monitoring data provide information which may be interpreted in the context of known mechanisms of action, and is directly relevant to assessment of dermal exposure and consequent health risk (Lehnert, 1980; Zielhuis and Henderson., 1986; see also Appendix E).

SECTION 8. HAZARD AND RISK ASSESSMENT OF CHEMICALLY CONTAMINATED SKIN

8.1. INTRODUCTION

In hazard assessment the potential of a chemical to cause adverse effects in man under well defined exposure conditions is evaluated (EEC, 1990). To assess the dermal exposure hazard the exposure of human skin to any chemical must be determined and the available procedures are described in Section 7. Hazard is then assessed by considering the available No Observable Effect Level (NOEL), preferably the dermal NOEL, and human systemic exposure data. In the first instance it can be assumed that the systemic exposure is equal to the dermal exposure, i.e. that there is 100% absorption. Only if this shows there is a hazard is there a need to quantify the absorption and to reassess the hazard.

It is evident that, with the current state of knowledge in the field of percutaneous absorption, hazard assessments should be performed on a case-by-case basis.

8.2. HAZARD IDENTIFICATION

Data from toxicity studies are needed to identify the hazards of chemicals. In the absence of any toxicity data the use of QSARs may give a preliminary identification of potential hazard (Berner and Cooper, 1987; Fiserova-Bergerova and Pierce, 1989; Fiserova-Bergerova *et al*, 1990). These approaches, at the moment, must be regarded as novel and need further development and validation. The most readily available data are those from acute, sub-chronic 28 and 90 day and chronic studies. These studies provide NOEL data. In the majority of cases no dermal data will be available as sub-chronic and chronic dermal dosing studies are technically more complex than oral studies. In this case the NOELs from oral studies must be considered relevant to dermal exposure and subsequent toxicity.

8.3. EXPOSURE ASSESSMENT

The optimum data for a hazard assessment of chemically contaminated skin are human systemic exposure data, but these are rarely available especially for industrial chemicals. In their absence dermal exposure data have to be used. Human dermal exposure may be estimated in the case of agrochemicals by use of mathematical models (BBLF, 1988; Ind. Verb, 1990). Methods to quantify human dermal exposure and the resulting systemic exposure are described in Section 7. In the first instance, the likely human exposure for new chemicals or formulations can be predicted from existing data on a product which is used in a similar manner (surrogate data).

The exposure assessment is relatively simple for products, such as cosmetics and barrier creams designed to remain in contact with the skin. It is more difficult to estimate skin exposure to rinse-off products. For occupational and accidental skin contamination the estimation of exposure is even more difficult (cf, Section 7); taking a worst case situation may offer the best estimate initially.

8.4. HAZARD ASSESSMENT

Comparing the highest dose of a chemical which does not lead to a toxic effect (NOEL) with the highest expected exposure allows calculation of the Margin of Safety (MoS). Thus

$$\frac{\text{NOEL}}{\text{Expected systemic exposure}} = \text{MoS}$$

For the general population a high ratio, e.g. >100, is an indication that the hazard is low; if the ratio is <100 problems may arise (Johannsen, 1990). However, lower ratios are frequently considered acceptable for specific work-related exposure situations.

While optimally, human hazard is assessed by comparing the systemic levels determined in man with those found in animal chronic toxicity studies and assuming that the animal model is relevant to man, such data are rarely available and a stepwise approach is recommended.

- The (sub-)chronic oral NOEL is compared with the estimated maximum human dermal exposure and 100% absorption is assumed. When there is an adequate MoS no further studies are necessary. When the MoS is inadequate step 2 is undertaken.

- An assessment of percutaneous absorption is made (cf Appendices C and D). Human data are preferred as animal data may lead to an overestimation of likely human absorption (cf Section 6 and Tables 5 and 6).

  Absorption flux or percentage absorption are used to estimate the human systemic exposure and hazard is re-assessed. When the MoS is insufficient the hazard remains high another step is required.

- A range of further pharmacokinetic, and probably, toxicity studies must be considered. The precise nature of these will be influenced by the chemical and its intended use. Amongst the studies might be a subchronic dermal study to establish a NOEL and plasma level determinations in test animals for comparison with levels found in other chronic or absorption studies.

When the hazard remains high a risk assessment should be made.

8.5. RISK ASSESSMENT

Risk assessment should not be confused with hazard assessment as it should include the probability of exposure occurring (EEC, 1990). In the case of consumer and similar products which are designed for direct application to the skin, the dermal exposure probability is equal to 1. In this case hazard is the same as risk.

The probability of dermal exposure to industrial chemicals during normal working practice or accidents should be estimated. When the probability of dermal contact with the chemical cannot be defined it is recommended that a worst case scenario (i.e. probability = 1) is applied or there may be a need to proceed directly to risk management (i.e. reduction of dermal exposure).

8.6. RISK MANAGEMENT

Risk management is, in relation to dermal exposure, the determination of action needed to eliminate, minimise or control the risk of percutaneous absorption. The risk management process has become formalised and the steps generally followed have been outlined (Krewski and Birkwood, 1987; Schettler *et al*, 1991). Guidance on the risk management is considered to be outside the scope of this document.

SECTION 9. CONCLUSIONS AND RECOMMENDATIONS

Percutaneous absorption is a significant route of uptake of many chemicals. The factors which influence percutaneous absorption are numerous. Thus, when an estimation of percutaneous absorption of a chemical is required for hazard and/or risk assessment, it is imperative that the conditions under which man will be exposed to that chemical are precisely defined. Knowledge of the exposure conditions enables the design of appropriate experiments and this, in turn, minimises the use of extrapolation factors which introduce uncertainties into the hazard assessment.

Percutaneous absorption is influenced by a number of variables, such as whether the contact is occlusive, the state of hydration of the skin, the presence of skin disease or physical damage, the type and duration of contact, the availability of the chemical to the skin surface, the application site, area, dosing regimen, temperature, circumstances of contact with the skin and the age of the subject. At present there is no single accepted and accurate method that will predict the amount of a substance which will be absorbed across the skin following a particular exposure; such data must be generated in experiments and this is clearly an area in need of further research.

Results from human volunteers studies are considered to be the most relevant for man but are rarely available. Data from *in vivo* animal studies are more common and results from these experiments normally overestimate human percutaneous absorption because of the higher permeability of the animal skin. Because additional information on tissue distribution, metabolism and excretion can be obtained from such animal studies these should be conducted in the species and strain used in the toxicity studies producing the NOEL used in the hazard assessment.

Available *in vivo* and *in vitro* data demonstrate that all animal skins are more permeable than human skin and that pig and monkey skin have permeability properties closest to human skin. When human skin is not available, pig skin is the preferred choice.

In vitro data with human and animal skin correlates well with *in vivo* data. Therefore regulatory authorities should accept data from *in vitro* studies (using *ex vivo* skin) which have been conducted using properly designed protocols. In considering these protocols the donor system must reproduce the hazard assessment conditions of human skin contact, the integrity of the skin membrane must be demonstrated and the receptor solution must provide skin conditions.

Artificial membranes are at present not an alternative to skin for prediction of percutaneous absorption. Membrane models based on cultured human skin hold promise for the future but need extensive validation.

The use of these model systems yields useful information but their limitations for the prediction of human percutaneous absorption must be understood. A prerequisite for confident extrapolation of such data is the standardisation of experimental procedures.

Percutaneous absorption data at present available have been derived from a range of experimental protocols and are too inconsistent to permit a general QSAR approach. Further studies and improvement of the models must be performed. This would allow the establishment of a quantitative data base relating chemical structure to skin permeability. This could be useful for the screening of new chemicals according to their percutaneous absorption rate potential.

Total dermal exposure has been defined as the amount of chemical in contact with skin for a specified time period. It is not only important to determine the amount of chemical in contact with the skin but also to state the duration of exposure because it influences the extent of the systemic exposure.

Although dermal exposure is simply defined it is often difficult to assess in practice. Both the amount of chemical in contact with the skin and the duration of exposure may be impossible to measure accurately. In such cases only a qualified estimate of dermal exposure can be made.

There are three approaches to the assessment of systemic exposure resulting from dermal exposure. Where no information on percutaneous absorption is available an assumption can be made that the dermally deposited dose is completely absorbed, i.e. systemic exposure = dermal exposure. Where percutaneous absorption data are available these can be used to convert dermal exposure into systemic exposure values, i.e. systemic exposure < dermal exposure. Biological monitoring can also be used to assess systemic exposure but it should be noted that systemic exposure measured by this method may not be a result of dermal exposure alone.

Hazard can be assessed by comparing available No Observed Effect Level (NOEL) data and human systemic exposure data. In the first instance 100% dermal absorption may be assumed and dermal exposure equal to systemic exposure. If this results in a hazard then percutaneous absorption, ideally determined in human volunteers, should be quantified and the hazard reassessed.

Ideally, for human hazard assessments for chemically contaminated skin, systemic levels in man should be compared with those found in animal chronic toxicity studies. Risk assessment must not be confused with hazard assessment as it includes the probability of exposure occurring. This probability is 1 for consumer products but must be estimated for industrial chemicals.

APPENDIX A GLOSSARY OF TERMS

BIOLOGICAL MARKER:
an indicator (usually obtained by analysis of tissue or observation of signs of an adverse effect) of susceptibility of an organism to exposure to a chemical substance.

BIOLOGICAL MONITORING:
a method for assessing absorption of substances into the body by measuring the substance or its metabolites in body fluids (usually blood or urine).

DERMAL AREA EXPOSURE:
the amount of a chemical in contact with a unit area of skin and this for a defined time period.

DERMAL AREA DOSE:
the amount of a chemical applied per unit area of skin.

HAZARD ASSESSMENT:
assessment of the potential for a substance to cause adverse effects with specified degrees of exposure.

HAZARD IDENTIFICATION:
the process of defining the inherent toxicity of a chemical and the degrees of exposure at which toxic effects become manifest.

PARTITION COEFFICIENT:
the ratio of concentrations of a chemical at equilibrium in two immiscible phases which are in contact. A wealth of data is available for the octanol-water partition coefficient.

PENETRANT:
the molecule of which the percutaneous absorption is being considered.

PERCUTANEOUS ABSORPTION:
the movement of chemicals from the outer surface of the skin to the systemic (circulatory) system.

PERCUTANEOUS FLUX:
amount of substance passing through a unit area of skin per unit of time.

QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR):
the quantitative relationship between parameters which correlate with chemical structure and to biological activity.

RISK ASSESSMENT:

assessment of the probability that a harmful effect will occur.

RISK MANAGEMENT:

the measures which may be appropriate to decrease or eliminate the risk.

SYSTEMIC EXPOSURE:

the total amount of a chemical (and metabolites and break-down products) absorbed through the skin after dermal exposure.

TOTAL DERMAL DOSE:

dermal area dose x exposed area.

TOTAL DERMAL EXPOSURE:

dermal area dose x exposed area for a specified time.

APPENDIX B COLIPA - USE LEVEL COSMETICS ESTIMATES

Comité de Liaison des Associations Européennes de l'Industrie de la Parfumerie, des Produits Cosmétiques et de Toilette rue de la Loi, 223 (Bte 2) - B1040 Brussels

Information on use levels of certain types of cosmetic products is given in Table B.1. The data were gathered in 1981 through a limited survey carried out amongst some COLIPA member-associations and member-companies.

Use levels of cosmetic products are subject to several factors, some of which will vary with time, such as age group, seasonal variations, local habits, fashion trends, disposable income, product innovation.

Because of these changing conditions, use levels of cosmetics should not be included in a document on guidelines for safety testing of cosmetic products and in particular their ingredients. They should rather be used in a case-by-case approach on the safety evaluation, once the results of testing, as advised in the guidelines, has become available.

The average quantity is the quantity used per application. For some product types, the quantity left on the skin is much less.

Table B.1 Typical Use Levels of Cosmetic Products

Product type	Average quantity per application (g)	Frequency of application
Mucous Membrance Contact		
Toothpaste	1.5	1-2/day
Mouthwash (read to use)	12	1-3/day
Eye make-up: powder	0.01	1-3/day
mascara	0.025	1/day
liner	0.005	1/day
Eye make-up remover (wiped off)	0.5	1-2/day
Lipstick	0.01	1-6/day
Non-Rinse Products		
Face cream	0.8	2/day
After-shave	1.2	1-2/day
General purpose cream	1[1]	1-2/day
Body lotion	7.5	1-2/day
Setting product	12	1-2/week
Hairspray (as sprayed)	10	1-2/day
Temporary hair dye	12	1-2/week
Toilet water	0.75	1-5/day
Talcum powder	2.5	1-2/day
Anti-perspirant/deodorant spray (as sprayed)	3	1-3/day
Make-up remover	2.5	1-2/day
Anti-perspirant/deodorant (others)	0.5	1-3/day
Nail products	0.25	2-3/week
Sun cream	8	2-3/day for 2 weeks/year
Sun lotion	10	1 week in winter only on face
Rinse-Off Products		
Shaving cream	2	1/day
Soap bar	0.8	3-6/day
Foam bath (undiluted)	17	1-2/week
Shower gel	10	1-4/week
Shampoo	12	1-7/week
Hair conditioner	14	1-3/week
Semi-permanent hair dye	30	8-18/year
Permanent hair dye (ready to use)	50	8-12/year

[1] mg/cm^2

APPENDIX C *IN VIVO* TEST METHODS FOR MEASURING PERCUTANEOUS ABSORPTION

C.1. ANIMAL STUDIES

C.1.1. INTRODUCTORY INFORMATION

Exposure to many chemicals can occur via the skin but the majority of toxicological studies performed in laboratory animals use the oral route of administration. This can present difficulties in making hazard assessments following dermal exposure. The rat percutaneous absorption study set out in this guideline provides the linkage necessary to extrapolate from oral studies when assessing the hazard of dermal exposure.

It is emphasised that animal skin is generally more permeable than human skin and therefore will overestimate human systemic exposure.

The protocol refers to the dermal absorption of chemicals in the rat. However, the basic principles may be applied to other species. This is a general protocol and regulatory guidelines may vary from this in different countries; these should be consulted.

Prerequisites

Physico-chemical properties of the chemical

Analytical procedure to identify the chemical

Definitions

DERMAL AREA EXPOSURE: the amount of a chemical in contact with a unit area of skin and this for a defined time period.

DERMAL AREA DOSE: the amount of a chemical applied per unit area of skin.

HAZARD ASSESSMENT: assessment of the potential for a substance to cause adverse effects with specified degrees of exposure.

PERCUTANEOUS ABSORPTION: the movement of chemicals from the outer surface of the skin to the systemic (circulatory) system.

SYSTEMIC EXPOSURE: the total amount of a chemical (and metabolites and breakdown products) absorbed through the skin after dermal exposure.

TOTAL DERMAL DOSE: dermal area dose x exposed area for a specified time.

TOTAL DERMAL EXPOSURE: dermal area dose x exposed area.

Principle of the Test Method. The test substance, preferably radiolabelled, is applied to the shaved skin of several groups of animals at an appropriate dose level. The compound is allowed to remain in contact with the skin for a fixed period of time. The dose site is protected from grooming by the animal by a suitable cover. After the chosen exposure period the cover is removed and the skin cleaned with an appropriate cleansing agent; the cover and the cleansing materials are retained for analysis and a fresh cover applied. The animals are housed prior to and after dosing for various time periods in individual metabolism cages and the excreta over these periods are collected for analysis. At the end of the time periods the animals are killed, blood is collected for analysis, the application site removed for analysis and the carcass is analysed for any unexcreted material. The samples are assayed by a suitable sensitive technique and the degree of percutaneous absorption estimated.

C.1.2. DESCRIPTION OF THE TEST PROCEDURE

Test substance. The test substance should, if possible, be radiolabelled in a metabolically stable position, preferably with ^{14}C and of a high radiochemical purity (preferably >98%). The radiolabelled compound may be diluted with non-radiolabelled substance. The specific activity and radiochemical purity of the test substance must be known. A non-radiolabelled compound may be used providing suitable validated assay procedures for the compound in the relevant samples exist and the metabolism of the compound is well characterised.

Experimental Animals.

Strain: The study will be carried out on rats, where possible of the same strain as those used to generate toxicological data.

Sex: Normally one sex would be sufficient. (The more sensitive sex based on toxicological data.)

Age: Young adult rats (typically 8-14 weeks) within the resting hair phase (dormant telogen).

Number of animals: 4 or 5 per exposure period.

Housing and environment: The animals should be acclimatised to the laboratory conditions for at least 3 days prior to transfer to individual metabolism cages for a further acclimatisation period of at least 1 day. The temperature of the room should be 22 ± 3°C and the relative humidity 30 to 70%. Where lighting is artificial the sequence should be 12hrs light and 12hrs

dark. During all periods of the study conventional laboratory diets and drinking water will be freely available.

Preparation of animals prior to dosing: Following the acclimatisation period, and at least sixteen hours prior to intended dosing, an area of hair on the shoulders and back will be removed. The area chosen must be at least $10cm^2$ and abrasions should be avoided. The animals will then be placed into their individual metabolism cages.

Preparation of the dose: The test substance should be prepared in a suitable vehicle to allow good contact with the skin. The stability of the substance under the proposed conditions of administration should be determined. Since permeation can be modified by the vehicle, careful consideration should be given to the choice of solvents in these studies. If possible, the commercial formulation should be used.

Dose concentration: A minimum of two concentrations should be used which span the range of formulation concentration which can be expected in human exposure. For example, in the case of an agrochemical, the concentrated formulation and field dilution should be used.

Test Conditions. Application of the Test Substance: A known amount of chemical should be applied to a defined area of skin. This should be protected by a non- occlusive covering unless the objectives of the study or the use pattern of the chemical preparation indicate otherwise.

Non-occlusive covering (one example given). Immediately prior to dosing, the animal is removed from its metabolism cage and a rubber O-ring, internal diameter 3-4 cm (or 2 smaller rings), is glued, using cyano-acrylate adhesive, to the skin over the proposed dose site. When the ring is securely attached to the skin the appropriate volume of the test substance should be applied, as a single dose, evenly over the skin within the O-ring. At least 4 animals should be treated per time-point. A standard volume of 10 µl per cm^2 should be applied although larger volumes can be used provided there is no significant run-off.

For solid formulations a dose level of up to $100mg/cm^2$ will be more than sufficient to cover the application site. A semi-occlusive cover, e.g. stiff nylon gauze glued to a second O-ring, is then glued over the O-ring which must be thick enough to prevent the gauze rubbing on the treated skin.

Occlusive covering: The nylon gauze may be replaced by non-porous tape.

Duration of exposure: this will be dependent upon the needs of the study but is likely to be 8 or 24 hours or continuous exposure.

Removal of unabsorbed dose: At the end of the exposure period each animal should be anaesthetised and the gauze removed and retained for analysis. The treated skin of all animals should be washed at least 3 times with an appropriate cleansing agent using cotton swabs. The cleansing agent should have minimal effects on the uptake of the test substance whilst effectively removing it from the skin. The skin should be dried with cotton swabs. All swabs and washings should be retained for analysis as one set per animal. A fresh gauze should be applied to the O-rings of those animals forming the later time point groups prior to their return to individual metabolism cages. Care must be taken to ensure that skin outside the "O" ring is not contaminated during the washing procedure.

Sample time points: Groups of animals should be killed at time points set according to the needs of the experiment.

Samples: Urine, faeces, $^{14}CO_2$, volatile ^{14}C-compound traps, cage washings, swabs and skin washings should be sampled for analysis. Samples of blood, major organs (if required), skin from the dose site and remaining carcass may be taken after the animals have been killed.

Analysis.

Radiolabelled compound: The radioactivity in each sample will be determined as follows:

- Urine, expired air trap solutions, plasma and washes/rinses: by direct liquid scintillation counting.
- Dose site skin, whole blood: combustion or solubilisation as appropriate followed by liquid scintillation counting.
- Tissues, carcass (whole minus dose site skin) and faeces: by homogenisation followed by combustion or solubilisation, as appropriate, and liquid scintillation counting.
- O-ring, protective gauzes and swabs: soaked in an appropriate solvent and sampled and analysed by liquid scintillation counting.

Non-radiolabelled Compound: All samples should be analysed by suitable validated procedures (which will record limits of determination and percentage recovery values for each procedure) specific for parent and/or metabolites.

C.1.3. RESULTS

For each animal the following determinations may be made. Results should be expressed in mg of active ingredient and/or degradation products, as appropriate.

- Quantity of the compound in/on the protective appliance.
- Quantity of the compound washed from the skin.
- Quantity of the compound at the site of application which cannot be removed by washing.

- Quantity of the compound excreted in the urine and faeces (and as $^{14}CO_2$ and/or volatile ^{14}C-compounds, if applicable).
- Quantity of the compound remaining in the carcass.
- Concentration of absorbed compound in blood.

From the above values the total amount absorbed at each time point (and % of applied dose absorbed) may then be calculated. Knowledge of the area of skin treated, i.e. area of skin enclosed by the O-ring, will enable an estimate of the absorbed dose to be expressed as unit weight of compound (mg) absorbed per cm^2 skin over the exposure period and any other subsequent times from a single application.

C.2. HUMAN VOLUNTEER STUDIES

C.2.1. INTRODUCTION

The aim of these studies in occupational health is to determine the extent of percutaneous absorption in man of chemicals to which skin exposure may occur. In the assessment of their hazard a comparison is sometimes made to the amount of chemical inhaled when man is exposed to safe atmospheric concentrations (Tuecker and Key, 1983). The applicability of these studies is severely restricted. In all cases, except perhaps for the development of pharmaceutical chemicals where the technique is more commonplace, practical and ethical considerations mean that *in vivo* human studies may address only specific questions. The conduct of human volunteer experiments is closely regulated (WMA, 1982; Declaration of Helsinki, 1983); the study protocols and necessary toxicological data must be submitted to a properly constituted ethical committee and approval obtained. Medical supervision of the studies is necessary.

Prerequisites
Physico-chemical properties of the chemical.

Toxicological data including sensitisation, irritation and systemic toxicity to assess the safety of the proposed exposure to the chemical to be tested.

Methods of analysis for the compound and/or its metabolites which should have been identified in pharmacokinetic studies. The use of radiolabelled compounds is possible in some countries but is subject to regulation (WHO, 1977; ICRP, 1977,1979; NRPB, 1988).

Definitions
DERMAL AREA EXPOSURE: the amount of a chemical in contact with a unit area of skin and this for a defined time period.

DERMAL AREA DOSE: the amount of a chemical applied per unit area of skin.

HAZARD ASSESSMENT: assessment of the potential for a substance to cause adverse effects with specified degrees of exposure.

PERCUTANEOUS ABSORPTION: the movement of chemicals from the outer surface of the skin to the systemic (circulatory) system.

SYSTEMIC EXPOSURE: the total amount of a chemical (and metabolites and breakdown products) absorbed through the skin after dermal exposure.

TOTAL DERMAL DOSE: dermal area dose x exposed area for a specified time.

TOTAL DERMAL EXPOSURE: dermal area dose x exposed area.

Principle of the Test Method. A known amount of test substance may be applied directly as liquid or solid to the skin or as vapour in an exposure chamber to an area of skin (e.g. an arm or the whole body). At the end of the exposure period the skin is rinsed to remove unabsorbed test compound (shower). The treated area of skin may be subjected to tape stripping to remove *stratum corneum* containing absorbed test material. Throughout the experiment blood, urine, expired air and faeces may be collected at regular time intervals and analysed for the presence of test compound and/or its metabolites.

C.2.2. DESCRIPTION OF THE TEST PROCEDURE

Test Substance. The test substance should preferably be >98% pure. In the case of radiolabelled material the radiochemical purity should be >98% and the radiolabel should ideally be in a metabolically stable position.

Test Preparation. This should normally be similar to a commercially available preparation and in a form expected to be in contact with skin. It may be undiluted or, if analytical sensitivity permits, at the "in use" dilution. Irritancy studies on the formulation should be performed in animals and then in man before the absorption study.

Selection of Subjects.

Numbers: A compromise is required between practicality and the need to account for variability: normally 5 volunteers would be adequate.

Age: Not less than 18 or more than 60 years, in order to mimic the population exposed.

Sex: Volunteers should normally be male.

Health: The medical status of all volunteers must be known and fully recorded. A medically qualified person should be included in the project team and should decide on any specific tests to be included in the medical examination. Drug addicts and those on chronic medication or with a high alcohol intake should be excluded due to the possibility of compromised metabolism.

Test Conditions. The volunteers should be informed of all procedures, known risks and areas of uncertainty and should sign a statement indicating that they have understood this information.

The volunteers in such a study should be housed under clinical conditions and be under medical supervision throughout the whole study.

Test site: This will depend on the amount of material to be applied. It is practical to apply from 100µl to 1ml to the forearm and up to 2ml to an entire arm. The use of a leg or the back may be contemplated but any inconvenience to the volunteer should be borne in mind.

Application: A fixed area of skin should be marked and the formulation evenly spread over the area or it should be exposed to vapour generated in an exposure chamber. The area will be normally governed by the sensitivity of the assay used to determine absorption, the concentration of the penetrant in the formulation and the anticipated rate of absorption. Often areas between $50cm^2$ and $1,000cm^2$ are used. The volume applied will be limited by the need to use a fixed area and not to have run-off. The area should preferably not be covered and every effort should be made to avoid inadvertant physical removal of the test material.

Duration of exposure: this will depend on the needs of the protocol but is likely to be 8 or 24 hours or continuous exposure.

Sample Collection and Analysis

Sampling period: The time blood and excreta samples are taken will depend on whether human oral data are available and what they show. Blood samples should, ideally, be taken when exposure commences, one or more times on the ascending portion of the curve, one or more times close to the peak and 3 or more times on the decline curve. Substances that are slowly absorbed and rapidly excreted may never reach high enough blood or plasma concentrations to obtain this ideal and may, indeed, only be detectable at and around the peak.

In the absence of oral data it is suggested that samples are taken at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60 and 72 hours. If samples can be analysed immediately, sampling may stop when concentrations fall below the limit of determination.

Urine collection should cover 12 hour periods except on the first day when collections should be over shorter periods. Volunteers should be encouraged to take fluids in reasonable quantities during the first 12 hours in order to give a good flow of urine. Sampling should ideally continue until the detection limit is reached. Urinary creatinine may also be measured as an indication of completeness of urinary collections.

Faeces, if sampled, should be put in a container labelled with the time and date of production.

Method of collection: Blood should be withdrawn from a site which is unlikely to be contaminated with test substance, e.g. the contralateral arm. Depending on the number of samples to be taken, a needle or canula can be used. It is reasonable to withdraw 5-20ml of blood per collection which should be transferred immediately to tubes; these should contain anticoagulant if the analysis is carried out on whole blood or plasma.

Urine should be collected in inert containers. Volunteers should be asked to empty their bladders into these containers at the end of each interval. The total volume collected at each time point should be recorded.

Faeces can be collected in polyethylene bags.

Skin rinsings should be collected as quantitatively as possible.

Tape strips from the treated skin should be extracted with suitable solvents to permit analysis of the parent compound residing in the *stratum corneum*.

Stability: Whatever the biological material to be analysed, it should be ascertained that the compound or metabolite can be extracted and analysed and that it is stable under the storage conditions contemplated. This can be done with spiked samples as a preliminary to running the study.

Results. For each subject the following determinations may be made, the results being expressed as mg of active ingredient and/or degradation products, as appropriate.

- Concentrations of absorbed compound or its metabolites in blood.
- Quantity of the compound excreted in the urine, faeces and expired air.
- Quantity of the compound rinsed from the treated skin area.
- Quantity of the compound removed by tape stripping.
- Quantity of the compound in/on the protective appliance which may have been used to protect the application site.
- In addition the findings should be given from the measurement of relevant biochemical markers in urine and blood.

From the above values the total amount of compound absorbed at each time point may then be calculated provided pharmacokinetic information relating blood and urinary levels to dose are available.

APPENDIX D *IN VITRO* TEST METHODS FOR MEASURING PERCUTANEOUS ABSORPTION

D.1. INTRODUCTION

A variety of methods are used to examine *in vitro* skin penetration; an overview of these is given here. Valuable advice on performing *in vitro* investigations can be found in the papers of Scott and Ramsey (1987), Gummer *et al*, (1987), Gummer (1989), and Hawkins (1990). An attempt to develop guidelines for bioequivalence testing of topical pharmaceutical preparations has been published by Skelly *et al*, (1987).

D.2. DIFFUSION CELLS

There are many types and designs of diffusion cells suitable for use in *in vitro* experiments. Cells must be fabricated from non-reactive materials like glass, stainless steel or transparent plastics (Plexiglas® or Teflon®). The *stratum corneum* of the skin faces the donor chamber and the dermal surface faces the receptor chamber fluid. By analysis of this receptor fluid, penetration of the test molecule through the skin is detected.

The existing horizontally or vertically orientated diffusion cells can be classified roughly into two different types, namely static or flow through cells. They are usually based on the original static Franz cell system (Franz, 1975) which are now most often used for applying a "finite dose". The skin is normally sandwiched between two halves of a glass chamber with the aid of O-rings (Hawkins and Reifenrath, 1984, 1986) or by overlapping the edge of the receptor compartment (Gummer *et al*, 1987).

Variations of the static diffusion cells can be classified as either having air/fluid phase or fluid/fluid phase chambers. In the air/fluid system the epidermal surface of the skin is uncovered and normally exposed to ambient conditions. Such exposure allows volatile solvents to evaporate and chemicals may be precipitated on the surface. With volatile substances, the air flow in the donor chamber should be carefully controlled because the evaporation rate influences the penetration values. These diffusion cells resemble more closely the normal situation because the skin is exposed to ambient conditions and is not excessively hydrated (Bronaugh and Stewart, 1985).

In fluid/fluid systems of the chemical passing from one stirred fluid phase (the donor side) through the skin into a second fluid phase (the receptor side) is determined. Side-by-side two chamber diffusion cells are widely employed (Dugard *et al*, 1984; Scott *et al*, 1986a); in these the skin is usually oriented vertically to the two diffusion chambers. Typically, the amount of chemical which penetrates is small compared to the dose in the donor compartment; therefore this type of *in vitro* measurement has been

referred to as the "infinite dose" technique. The fluid/fluid phase diffusion system represents occlusive exposure conditions.

In such an "infinite dose" experimental design non-physiological alterations of the skin may be caused by excessive hydration or by solvent induced deterioration of the *stratum corneum*. The results of these investigations have, therefore, to be carefully evaluated when used for hazard assessment. This type of diffusion cell is probably not suited to the measurement of absorption rates after a period of 24h since structural deterioration of the skin permeation barrier would lead to erroneous conclusions. In addition, skin surface temperature higher than 30°C must be avoided (van der Merve *et al*, 1988). Despite these limitations this diffusion cell arrangement is useful for the measurement of fluxes and partition and diffusion coefficients but is not recommended for measuring absorption from various formulations.

In static diffusion cells the small volumes of receptor solution drawn for analysis are replaced manually. In flow-through cells these volumes are automatically replenished (Bronaugh and Stewart, 1985). If low solubility of the penetrant in the receptor phase is a problem, the large volume and the continuous flow of the effluent may increase the amount of lipophilic chemical dissolved and thus improve the reliability of the data (Cooper, 1984).

Recommendations for diffusion cell design were summarised by Gummer *et al*, (1987) and Gummer (1989). The donor compartment should allow easy access for delivery of the penetrant to the skin, should be stirred where possible and necessary, should be temperature controlled and allow the control of evaporation of volatile vehicles and penetrants. The receptor compartment should also be temperature controlled, have sufficient volume to maintain perfect skin conditions and stirred without obvious formation of boundary layers.

D.3. SKIN MEMBRANES

In *in vitro* skin penetration experiments, freshly excised skin (from laboratory animals or human surgery) or cadaver skin is used. Subcutaneous fat and any attached muscles should be carefully removed from the prepared skin areas. Human cadaver skin stored in air-tight bags in the deep freezer up to 12 months can be used provided the integrity of the barrier is checked using ^{3}H-water as a probe (Bronaugh and Stewart, 1986; Bronaugh, 1990).

Full or as split-thickness skin can be used. Split-thickness skin is especially recommended in animals like the rat, guinea pig, monkey and pig and in man where the thickness of whole skin is about 1mm or more (man). Mouse skin, which is less than 500µm thick, can be used as a whole. The dermatome is a widely used technique for preparation of split thickness skin of about 400µm. It can be used with hairless and hairy skin and it does not adversely effect the viability of the membrane (Bronaugh, 1990).

Dermatomed skin must be used with highly hydrophobic compounds, where the aqueous dermis may represent a high diffusion barrier not apparent *in vivo*.

For separation of epidermis and dermis several mechanical, thermal and chemical techniques have been described. The epidermis can be readily removed with forceps after placing the skin samples on a hot plate for 2 minutes at 50°C or in beaker of water at 60°C for 30 - 60 seconds (Kligman and Christophers, 1963). This technique is particularly useful with human and hairless animal skin. Chemical separation by soaking skin samples in 2 molar solutions of various anions like bromide, thiocyanate and iodide, seems also to be effective in haired skin. Enzymatic separation using incubation with proteases like dispase, trypsin, pancreatin or a bacterial collagenase (Skerrow, 1980; Bronaugh, 1990) is also effective. Protease incubations have also been used for preparation of isolated sheets of the *stratum corneum* of human cadaver skin.

D.4. RECEPTOR FLUID

The receptor fluid under the dermal side of the skin membrane has to fulfil two functions. It should maintain the integrity of the skin membrane during the experiment and it should act as an effective acceptor for the penetrating compound. For water soluble compounds, physiological saline buffered to pH 7.4 is recommended as receptor phase. In case of lipophilic compounds (octanol/water partition coefficient $\geq 10^3$) the receptor phase needs to be altered to increase its ability to solubilise lipophilic molecules: the addition of non-ionic surfactants like polyethylene glycol oleyl-ether (6% in saline), methanol, ethanol, polyethyleneglycol, serum or albumin to the buffer solution is described in the literature (Bronaugh and Stewart, 1985; Scott, 1987; Bronaugh, 1989). It is necessary to ascertain that the receptor fluids used do not alter the permeability properties of the skin.

D.5. SKIN METABOLISM

Skin is capable of metabolising endogenous and exogenous substances. The biotransformation reactions in the skin comprise metabolism, e.g. by oxidation, reduction and hydrolysis (phase I) and conjugation e.g. with glucuronic and sulphuric acid, glutathione and methylation (phase II) (Pannatier *et al*, 1978; Noonan and Wester, 1989). Although the spectrum of reactions in the skin is similar to that in liver, the activity is much lower in the skin (Mukhtar and Kahn, 1989; Taeuber, 1989). To suppress bacterial growth and metabolism an antibiotic is added to the receptor fluid.

In vitro percutaneous absorption studies with viable skin can indicate if a test substance may be toxified or detoxified during skin permeation. The various factors like area dose, vehicle, concentration, skin condition, mode of application (open versus occlusion) etc. influencing percutaneous absorption, and probably the extent of skin metabolism, make the "finite dose" technique best suited to mimic *in vivo* metabolic conditions. Quantitatation of skin metabolism findings has to be interpreted with caution.

Further investigations are necessary to assess how well *in vitro* absorption experiments with viable skin predict *in vivo* absorption in man.

D.6. TEMPERATURE

Because the absorption process relies on diffusion of molecules, the rate can be altered by changes in temperature (Hawkins and Reifenrath, 1984). Skin should be maintained at a constant temperature; 30°C ±1°C is recommended in Franz type designs as this approximates normal skin temperature.

D.7. APPLICATION OF TEST SUBSTANCE

The "finite dose" technique allows varying amounts of material to be applied to the skin surface. In this way the experiment can provide data from a range of preparations such as powders, undiluted formulation, aqueous dilutions, and large and small volumes. The precise dose will inevitably depend on the data required. Solids or samples with a high viscosity can be applied to the skin on a weight basis using a rod; liquids are applied usually by volume using a syringe.

D.8. SAMPLING AND ANALYSIS

Samples of the receptor fluids, of a size determined by the assay procedure and its sensitivity, can be taken during the exposure period. These are analysed by liquid scintillation counting if radiolabelled penetrants are used or by GLC/HPLC if unlabelled chemicals are used (Loden, 1990).

It is recommended that the concentration of the chemical remaining in the skin at the end of the experiment is determined. The distribution of the chemical in different skin layers can be evaluated by several methods, e.g. tape stripping, horizontal sectioning or autoradiography (Schaefer *et al*, 1982; Gummer, 1989; Hall and Shah, 1990).

The effects of decontaminating the skin after a defined period of exposure can be studied by washing the skin surface with e.g. saline and detergent solution.

D.9. RESULTS

The absorption profile should be characterised from immediately after application of a compound up to a minimum of 24 hours. If the data allow the definition of lag times and calculation of absorption rates these should be presented (Dugard, 1987; Ravis, 1990).

The slope of the regression line through the linear portion of the curve represents the amount of substance penetrated through the treatment area per time unit, dM/dt; the extrapolated intercept on the time axis represents the lag time L (cf. Figure 2).

From Fick's First Law of Diffusion a permeability coefficient P can be calculated :

$$P = \frac{dM/dt}{A \cdot C}$$

where

P	= permeability coefficient in length/time
dM/dt	= substance flux in mass units per time
A	= surface area of skin exposed ($length^2$)
C	= concentration of test substance in the donor solution in mass units per volume

From the lag time L the diffusion coefficient D is obtained:

$$D = \frac{d^2}{6L}$$

where

D	= diffusion coefficient in $length^2/time$
d	= thickness of membrane (horny layer) in length units
L	= lag time

While the "infinite dose" technique has been of great value in elucidating the physico-chemical concepts of percutaneous absorption, this methodology is only of limited value for predicting skin absorption and systemic load *in vivo.* For this the "finite dose" technique was developed in which the skin surface can be exposed to solutions, ointments, powders etc. similar to the exposure *in vivo.* Cumulated amounts penetrating a certain area of skin in a defined time can be directly obtained from cumulative amounts versus time curves.

From these plots a time averaged substance absorption rate (flux J) can be derived according to :

$$J = \frac{M\ (o\text{-}t)}{A \cdot t}$$

where

M(o-t)	= cumulated amount absorbed within exposure time t in mass units
A	= skin area exposed to test agent in cm^2
t	= exposure time *in vitro* (h)

From such kind of experiments the total *in vivo* body absorption may be estimated. An untested, but possibly useful approach is to use the following equation which takes account of different skin permeabilities in different regions of the human body:

$$E_{o-t} = J\sum_{i=1}^{i=n} a_i \,.\, P_i \qquad (4)$$

where

E_{o-t}	=	total systemic exposure within an exposure time t
J	=	time averaged *in vitro* flux (exposure time t)
a_i	=	area of exposure in region i
P_i	=	penetration index of region i relative to penetration index of skin site used in the *in vitro* experiment

APPENDIX E METHODS FOR DERMAL EXPOSURE ASSESSMENT

E.1. DERMAL EXPOSURE

E.1.1. Techniques for measuring dermal exposure to areas other than hands

It has been shown that contact with the skin is a major route of exposure. Furthermore, exposure to the forearms and hands may be 90% or more of the total dermal exposure.

While the importance of assessing the amount of skin deposition is widely accepted, there is some concern about the accuracy of the measurement methods currently employed. The consensus is that present methods give only a rough estimate of potential dermal exposure even when carefully done by an experienced research team (NACA, 1985; US-EPA, 1984; WHO, 1982).

Patch Technique. Dermal exposure is usually estimated by the patch technique pioneered by Durham and Wolfe (1962). Up to 12 absorbent pads (often 10 x 10cm surgical gauze pads or cellulose patches) are attached at various locations on a worker's body, outside the clothing, underneath the clothing, or both. Outside patches monitor the amount of chemical impinging on clothing while inside ones indicate the amount that could reach the skin while operations are carried out using a chemical. Differences between the amounts of chemical on inside and outside patches give an estimate of the protective effect of the clothing. The quantity of substances retained on a particular pad is considered to represent the dermal exposure to that region of the body to which that pad is attached. The mass of contaminant found on the pad by chemical analysis is multiplied by a factor equal to the ratio of the area of that region of the body to the area of the pad. Total dermal exposure is calculated by adding these estimated exposures for each skin region. Many investigators consider that the technique overestimates exposure.

The patches may consist of a pad of gauze or alphacellulose material of known size with an impervious backing such as vinyl, polyethylene, glassine paper, etc. Equivalent devices may be substituted where appropriate. Exposure patches can be attached either outside or inside the clothing or both. The location of the pads on the body will vary with the objectives of the study. The inner patches can be attached to underwear or to the worker's skin and the outer patches to washable or disposable coveralls, or other garments worn by those exposed. The patches can be attached by velcro adhesive strips, pressure sensitive adhesive strips, safety pins or staples. Attachment directly to skin is ideal but may be unconfortable. Popendorf and Leffingwell (1982) have had some success using surgical tape.

To prevent the outer patch covering the inner patch and to prevent bias due to one-sided exposure, the inner and outer patches can be randomly attached to alternate sides of a worker's body in the following locations: chest (breast area), back (center), thighs, lower legs (just below knees), upper arms and forearms. Two additional patches can be attached to the front and back of headgear to detect exposure to the head and neck area.

Care has to be taken to avoid non-work related contamination of the patches. This can occur in several ways and it should be prevented by instructing workers how to avoid touching, handling, or removing the patches. Personnel conducting the study must avoid contamination when applying, removing, and storing the patches. The absorbent surface should not be touched and patches should be isolated individually during storage. Disposable surgical gloves and forceps are frequently used to minimise contamination during their handling. Plastic bags or wide mouth sample bottles can be used to separate the individual patches for transport to the laboratory.

Disposable Coveralls. Some investigators have used disposable protective coveralls to assess dermal exposure. This is a conservative extension of the patch technique. The coveralls are used as the collection media and cover the major portion of the body so the error associated with extrapolation from monitored to unmonitored regions inherent be attributed to the patch technique. The use of disposable coveralls for measuring dermal exposure is addressed in more detail in the World Health Organisation protocol (WHO, 1982).

Solvent Rinse Technique. Keenan and Cole (1982) reported on the construction and use of an aerosol solvent delivery system to measure dermal exposure to aromatic hydrocarbons. This system may also have some application in the measurement of dermal exposure to other substances. In principle it is similar to the patch technique, in that only a small area of a body region is actually sampled and the results have to be extrapolated to estimate exposure for the entire region. The advantage of this system is that a greater number of samples can be collected over a single region and therefore that findings are more representative of the average exposure to that region. There are also advantages in analytical sensitivity and ease of use. A criticism of the skin rinse techniques is that they remove only the unabsorbed or slowly absorbed portion of material deposited on skin and so may underestimate exposure to compounds that are rapidly absorbed.

E.1.2. Techniques for Measuring Hand Exposure

Hand Wash. Hand contamination can be estimated by analysing substance present in hand rinses or in thin cotton absorbent gloves. The hand rinse removes only the unabsorbed substance so that for materials that are rapidly absorbed, the technique may underestimate exposure (Popendorf and Leffingwell, 1982); there is also a possibility that some solvents used to rinse the hands may enhance absorption of the chemicals.

The hand wash, as described by Durham and Wolfe (1962), is a technique commonly employed for measuring hand exposure. Each hand is washed in a polyethylene bag containing a suitable solvent. Ethanol is most commonly used, although distilled water and other solvents have also been used. Where techniques involve transfering solvent, care has to be taken to prevent the loss of chemical. For example some investigators have used a technique in which approximately 150ml of a solvent is applied from a wash bottle in two steps: a 30-second wash followed by a 30-second rinse. The solvent is caught in an stainless steel pan from which it is poured into a glass sample bottle. Then, the pan is rinsed with an additional 50ml of solvent to remove any remaining chemical residue.

Cotton Absorbent Gloves. Lightweight cotton absorbent gloves have been used by several investigators to measure hand exposure. If protective gloves are worn the absorbent gloves are worn inside them. Many investigators consider analysis of cotton gloves can result in exceptionally high estimates of exposure since they tend to absorb more liquid than would normally be expected to adhere to the skin; exposure estimated from glove data has, at times, exceeded the level that would cause obvious toxicological effects, while, in practice, no such effects have been seen.

E.1.3. Calculation of Dermal Exposure

Determine the mass (µg) of analyte on each patch and divide by the surface area of the patch (cm^2) to obtain $\mu g/cm^2$. Multiply this by the surface area (cm^2) of the body region represented by the patch. Several estimates of the size of these areas haven appeared in the literature (Berkow, 1931; Parker and West, 1973; Popendorf, 1976), differing from each often only slightly.

Representative values from Berkow (1931) are:

Face	$825cm^2$
Front of the neck	$150cm^2$
Back of the neck	$100cm^2$
Hands	$800cm^2$
Forearms	$1,200cm^2$
Thighs	$3,500cm^2$
Calves	$2,300cm^2$

Add the exposures for all the body areas of interest. For a fully clothed worker (body, legs, arms and head covered), the exposed areas are likely to be the face, the neck and upper thorax and hands. If protective gloves are worn, hand exposure values should be taken from samples collected under the protective gloves. Divide the total dermal exposure (µg) by the total amount of substances used by the worker during the exposure period. The dermal exposure is then expressed in µg/kg substance used.

If patches underneath clothing are included in the exposure determination, in recognition that there may be some penetration of substances through clothing, they are treated in the same manner.

Substances deposited on the hands are determined by hand washes, by deposition on absorbent gloves or by other means. The exposures are added to the total exposure.

It is generally recognised that all substance deposited on the skin may not be totally absorbed. Where an estimate of the degree of penetration is available from dermal penetration studies or from analogy with similar compounds with known degrees of penetration. The systemic dose is estimated by applying the appropriate dermal absorption factor to dermal exposure. In the absence of penetration data 100% absorption has to be assumed. Examples of how exposure data could be used to estimate dermal exposure doses are listed in NACA (1985).

E.2. BIOLOGICAL MONITORING

While complex, biological monitoring is the most direct way to assess body burden. Extrapolation from exposure data alone can result in erroneous estimates of body burden unless the rate of dermal absorption, surface area of contact and duration of contact are known. Biological monitoring, where practicable, is the most effective means for measuring total dose. It has the advantage that dermal penetration need not be determined and the problems associated with the interception of substances by patches and extrapolation to anatomic regions of the body can be avoided.

To provide a quantitative measure of systemic exposure, biological monitoring requires adequate supporting data to define the quantitative relationship between systemic dose and the amounts and rates of excretion of the substances and their metabolites. Specific and sensitive methods are required for determination of the parent compound and/or metabolites (doses) in appropriate biological material, as in detailed knowledge of the pharmacokinetics of the chemical in man. Biological monitoring i.e. measurement of physiologic and/or pharmacologic effects, e.g. inhibition of blood acetylcholinesterase, Met-Hb-values, can also be useful in defining dermal exposure (Lehnert, 1980; Zielhuis and Henderson, 1986; Leung and Paustenbach, 1988).

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No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No. 6	Acute Toxicity Tests, LD_{50}(LC_{50}) Determinations and Alternatives
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No. 11	Eye Irritation Testing
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity)
No. 13	DNA and Protein Adducts: Evaluation of their Use in exposure Monitoring and Risk Assessment
No. 14	Skin Sensitisation Testing
No. 15	Skin Irritation
No. 16	Mutation Research, Special Issue: Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals
No. 19	Respiratory Sensitisation
No. 20	Percutaneous Absorption

JACC REPORTS

No.	Title
No. 1	Joint Assessment of Commodity Chemicals, Melamine
No. 2	Joint Assessment of Commodity Chemicals, 1,4-Dioxane
No. 3	Joint Assessment of Commodity Chemicals, Methyl Ethyl Ketone
No. 4	Joint Assessment of Commodity Chemicals, Methylene Chloride
No. 5	Joint Assessment of Commodity Chemicals, Vinylidene Chloride
No. 6	Joint Assessment of Commodity Chemicals, Xylenes
No. 7	Joint Assessment of Commodity Chemicals, Ethylbenzene
No. 8	Joint Assessment of Commodity Chemicals, Methyl Isobutyl Ketone
No. 9	Joint Assessment of Commodity Chemicals, Chlorodifluoromethane
No. 10	Joint Assessment of Commodity Chemicals, Isophorone
No. 11	Joint Assessment of Commodity Chemicals, (HFA-132b) 1,2-Dichloro-1,1-Difluoroethane
No. 12	Joint Assessment of Commodity Chemicals, (HFA-124) 1-Chloro-1,2,2,2-Tetrafluoroethane
No. 13	Joint Assessment of Commodity Chemicals, (HFA-123) 1,1-Dichloro-2,2,2-Trifluoroethane
No. 14	Joint Assessment of Commodity Chemicals, (HFA-133a) 1-Chloro-2,2,2-Trifluoromethane
No. 15	Joint Assessment of Commodity Chemicals, (HFA-141B) 1-Fluoro 1,1-Dichloroethane
No. 16	Joint Assessment of Commodity Chemicals, (HCFC-21) Dichlorofluoromethane
No. 17	Joint Assessment of Commodity Chemicals, (HFA-142b) 1-Chloro-1,1,Difluoroethane
No. 18	Joint Assessment of Commodity Chemicals, Vinylacetate
No. 19	Joint Assessment of Commodity Chemicals, Dicyclopentadiene
No. 20	Joint Assessment of Commodity Chemicals, Tris-/Bis-/Mono-(2-ethylhexyl)phosphate
No. 21	Joint Assessment of Commodity Chemicals, Tris-(2-butoxyethyl)-phosphate
No. 22	Joint Assessment of Commodity Chemicals, Hydrogen Peroxide

TECHNICAL REPORTS

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man
No. 6	Formaldehyde Toxicology: an Up-Dating of the ECETOC Technical reports 1 and 2
No. 7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8	Biodegradation Testing: An Assessment of the Present Status
No. 9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11	Ethylene Oxide Toxicology and its Relevance to Man : An Up-Dating of ECETOC Technical Report n°5
No. 12	The Phototransformation of Chemicals in Water : Results of a Ring-Test
No. 13	The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on the Environment
No. 14	The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on Human Health
No. 15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16	A review of Recent Literature on the Toxicology of Benzene
No. 17	The Toxicology of Glycol Ethers and its Relevance to Man : An Up-Dating of ECETOC Technical Report n°4
No. 18	Harmonisation of Ready Biodegradability Tests
No. 19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20	Biodegradation Tests for Poorly-Soluble Compounds
No. 21	Guide to the Classification of Carcinogens, Mutagens and Teratogens Under the 6th Amendment
No. 22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24	The EEC 6th Amendment : Prolonged Fish Toxicity Tests
No. 25	Evaluation of Fish Tainting
No. 26	The Assessment of Carcinogenic Hazard for Human Beings Exposed to Methylene Chloride
No. 27	Nitrate and Drinking Water
No. 28	Evaluation of Anaerobic Biodegradation
No. 29	Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico- Chemical Properties, Tonnage and Use Pattern
No. 30(4)	Existing Chemicals : Literature Reviews and Evaluations
No. 31	The Mutagenicity and Carcinogenicity of Vinyl Chloride : A Historical Review and Assessment
No. 32	Methylene Chloride (Dichloromethane) : Human Risk Assessment Using Experimental Animal Data
No. 33	Nickel and Nickel Compounds : Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34	Methylene Chloride (Dichloromethane) : An Overview of Experimental Work Investigating Species, Differences in Carcinogenicity and their Relevance to Man
No. 35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36	Biomonitoring of Industrial Effluents
No. 37	Tetrachloroethylene : Assessment of Human Carcinogenic Hazard
No. 38	A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39	Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No. 40	Hazard Assessment of Chemical Contaminents in Soil
No. 41	Human Exposure to N-Nitrosamines, Their Effects and a Risk Assessment for n-Nitrosodiethanolamine in Personal Care Products
No. 42	Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43	Emergency Exposure Indices for Industrial Chemicals
No. 44	Biodegradation Kinetics
No. 45	Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis
No. 46	EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47	EC 7th Amendment: 'Toxic to Reproduction' - Guidance on Classification
No. 48	Eye Irritation: Reference Chemicals Data Bank
No. 49	Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50	Estimating the Environmental Concentrations of Chemicals Using Fate and Exposure Models
No. 51	Environmental Hazard Assessment of Substances
No. 52	Styrene Toxicology Investigations on the Potential for Carcinogenicity
No. 53	DHTDMAC: Aquatic and Terrestrial Hazard Assessment. CAS No. 61789-80-8