EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate E - Food Safety: plant health, animal health and welfare, international questions
E1 - Plant health

Sanco/222/2000 rev. 7
19 March 2004

Guidance Document on Dermal Absorption

This document has been conceived as a working document of the Commission Services which was elaborated in co-operation with the Member States. It does not intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State within the implementation prerogatives under Annex II, III and VI of Commission Directive 91/414/EEC, nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

1 Introduction

This document intends to provide guidance to notifiers and Member States on the setting of dermal absorption values to be used in risk assessment for users of plant protection products reviewed for inclusion in Annex I of Directive 91/414/EEC. Inclusion of active substances in Annex I to Directive 91/414/EEC (Article 4(1)(a) and (b)) is possible only if the products containing them can be used with acceptable risk to humans (i.e. operators, workers, bystanders). Evaluation of risk to these groups is essential for the issue of authorizations for release onto the market (Article 5(1)(b) of the Directive).

> To provide a reliable framework for the review process for decision making on Annex I inclusion of an active substance and to avoid undue delays, the current version of this guidance document should therefore only be used for the review of existing active substances notified in the third phase of the review programme according to Regulation 451/2000[1] and subsequent phases. For new active substances, the document should be implemented with dossiers for active substances submitted from 1 January 2005. However, some flexibility may still be necessary during a transitional period. Decision making should take into consideration that certain data requirements which are now triggered, may not have been obvious to applicants or notifiers at the time of their notification or dossier submission. Likewise, if this appears justified in individual cases and facilitates decision making, the updated guidance may be considered also for substances submitted in earlier phases of the review programme.
> For ongoing evaluations where no measured data are available, a default value of 10% may still be used in the risk assessment by the rapporteur Member State for the purpose of deciding on 'one safe use' in accordance of article 5(1) unless there are clear indications that 10% would be unrealistically low (e.g. based on physical chemical properties of the active substance). For such substances the endpoint list shall mention that the dermal absorption is "not determined" and the need for further dermal absorption data will be identified as a confirmatory data requirement and Member States will have to use such data at Member State level when applying the Uniform Principles.

The dermal route is the main exposure route for most pesticides for operators applying them (Wolfe, 1976) as well as for workers and bystanders (Ross, 1992). In the absence of experimental data, the occupational exposure is based on models. These models must permit a representative evaluation of human exposure in the field using application techniques and equipment representative of the use in question. The operator exposure models currently in use are UK POEM and BBA (in future EURO POEM) each calculating external dermal and inhalation exposure. Subsequently, for risk assessment these external exposure data are to be compared with toxicity data, i.e. the AOEL (Acceptable Operator Exposure Level). The AOEL is by default defined as an internal value and expressed in mg/kg/day (EC draft guidance document, 2001). To compare the external exposure with the internal AOEL, the external exposure data have to be turned into internal levels. For this purpose, knowledge of dermal absorption is essential. Directive 91/414/EEC indicates the circumstances in which dermal absorption studies are required. These studies should be performed in accordance with OECD guidelines 427 and 428 and associated guidance document (OECD, 2000a, b, c).

In the present document a brief overview of dermal absorption is given, including information on factors that may influence dermal absorption. This document provides a stepwise approach for derivation of default values for dermal absorption, as well as guidance on how to conduct relevant dermal absorption studies and how to use the data from these studies. In addition, a tiered approach for occupational risk assessment is presented in which dermal absorption and exposure assessment are integrated.

[1] OJ L 55, 29.02.2000, p.25

2 An overview of dermal absorption

2.1 Introduction

The dermal absorption is one of the determining factors in assessment of the internal exposure of pesticide workers and operators.
The establishment of a value for dermal absorption may be performed by use of a tiered approach from a worst case to a more refined estimate (De Heer, 1999). In a *first tier* of risk assessment, a worst case value for dermal absorption of 100% may be used for external dermal exposure in the absence of relevant information (Benford, 1999). An estimate of dermal absorption could be made by considering other relevant data on the substance (e.g., molecular weight (MW), log P_{ow} and oral absorption data) (*second tier*) or by considering experimental dermal absorption data (*third tier*).

Dermal absorption, the process by which a substance is transported across the skin and taken up into the living tissue of the body (EPA, 1992), is a complex process. The skin is a multilayered biomembrane with particular absorption characteristics. It is a dynamic, living tissue and as such its absorption characteristics are susceptible to constant changes.
Upon contact with the skin, a compound penetrates into the dead *stratum corneum* (when not evaporating or being rubbed off from the exposed area) and may subsequently reach the viable epidermis, the dermis and the vascular network. During the absorption process, the compound may be subject to biotransformation (for review see Noonan and Wester, 1989). The *stratum corneum* provides its greatest barrier function against hydrophilic compounds, whereas the viable epidermis is most resistant to highly lipophilic compounds (Flynn, 1985).

Dermal absorption is influenced by many factors e.g. physicochemical properties of the substance, vehicle, occlusion, concentration, exposure pattern, skin site of the body, etc. (for review see Howes, 1996; Schaefer and Redelmeier, 1996; ECETOC, 1993). It is generally tested in studies according to methodologies described by international platforms (OECD, 2000a,b,c; EPA, 1996, 1999; ECETOC, 1993; Howes, 1996; Diembeck, 1999). These documents provide a certain amount of standardisation and thereby improve the comparison of data between studies. These guidelines, however, give only a general description of the experimental design, whereas a proper study protocol (be it *in vivo* or *in vitro*) should take the anticipated exposure conditions into account. In the next sections (2.2 through 2.4) a brief overview is given of factors that influence the dermal absorption.

2.2 The studied tissue

Skin structure differs from one species to another (for instance, in the epidermis the stratum corneum is thicker in species without hair), between different strains of the same species and even within the same species (for instance, differences in absorption for the various parts of the body). Within the dermis, the number and density of collagen and elastic fibers and the density and physiology of the vascular system vary from one species to another. Certain species have sweat glands, others do not. In case of *in vitro* experiments it should be realised that the blood vessels and nerve fibers are not functional. Three types of skin membranes can be prepared for *in vitro* experiments: epidermal membranes (thickness of approximately 0.1 mm, prepared by heat separation, chemical or enzymatic separation), split-thickness skin (thickness of 0.2 - 0.5 mm prepared using a dermatome) and full-thickness skin (thickness of 0.5 - 1.0 mm). Since the main barrier function of the skin is located in the *stratum corneum*, all three membrane types have been used for absorption studies. A possible disadvantage of full-thickness skin is that lipophilic compounds may be retained in the dermis instead of entering into the receptor fluid. On the other hand, epidermal membranes are more fragile and sometimes overestimate human *in vivo* skin absorption (Van de Sandt, 2000).
Certain inter-species differences are well documented (Feldman, 1970 ; Bartek, 1972, Maibach, 1989 ; Bronaugh, 1987, 1990). According to Brandau and Lippol (1982) skin permeability across the species is in the following descending order: rabbit > rat > guinea-pig > mini-pig > Rhesus

monkey > man. Scott (1991) have demonstrated that skin permeability could be related to inter-species differences in skin structure, but only with the relatively slowly absorbed test penetrants. Rat skin is typically two to ten times more permeable than human (ECETOC, 1993). However, occasionally rat skin permeability resembles that of human skin (Ross, 2000).
A further factor which should be taken into account because of its influence on dermal absorption is the location of the contact between product and skin (for example the skin of the scrotum is twelve times more permeable than that of the fore-arm and the forehead is more permeable than the cheeks) (Wépierre, 1970). In this respect it is noted that, human skin membranes are usually prepared from abdominal or breast skin, while for obtaining animal skin the commonly used sites are the flank and back (rat), or the flank and ear (pig).

2.3 Active substance properties affecting penetration

Physical and chemical properties have a decisive influence on the penetration of molecules through the skin. The most important of these seem to be :

- liposolubility (usually maximal when log Pow is between +1 and + 2).
- molecular weight (molecules with low MW pass more easily).
- electronic structure and dissociation constant (pK_a): highly ionised products do not penetrate very much.
- the nature of the carrier and the dilution factor of the substance is decisive (polar or non-polar): non-polar carriers increase penetration.
- presence in the molecular structure of certain special radicals favouring or inhibiting penetration.
- water distinctly favours penetration, as does sodium chloride, for instance.

2.4 Experimental conditions

The experimental design and test conditions used may significantly affects the results obtained (Kemppainen, 1990).

In vivo and *in vitro* experiments have demonstrated there is an inverse relation between concentration (area dose) and percentage of absorption. At low concentrations the absorbed test substance expressed as percent of applied dose per time interval is in general higher than the percentage absorption at high concentrations. As a consequence, there is no standard absorption percentage for a given substance. Therefore, dermal absorption studies should be done at different concentrations as a function of the planned agricultural practice.
These considerations indicate that it may not be necessary to request skin absorption studies on the active substances themselves as absorption can vary with solvent used (Southwell, 1983 ; Bronaugh, 1986; Lee, 1994) and dilution. But there must be studies with the preparation at doses including the undiluted preparation and the preparation as diluted to recommended concentrations for uses in the field (Kemppainen,1990; Bronaugh, 1991b; Scott, 1993), or if not, at the strongest dilution. Since different solvents may mean different absorption percentages there should be a new determination of absorption percentages when a significantly different preparation is proposed.

Different absorption percentages can then be established for preparations :

- The estimated absorption % with the undiluted preparation can be used to estimate absorption during the mixing/loading phase. Although it should be noted that the

concentration during mixing/loading might also be lower (which might result in an underestimation of the internal dose).

- The estimated absorption % with the preparation diluted in water to the minimum recommended use concentrations for field applications can be used to estimate absorption during the spraying phase.

3 Studies on dermal absorption

3.1 *In vitro* studies

The test should be carried out in accordance with "OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* method" (OECD, 2000a) and the Draft OECD Guidance Document for the Conduct of Skin Absorption Studies (OECD, 2000c).
In the case of agrochemicals the exposure period as well as the testing conditions and the concentration examined should reflect the anticipated occupational exposure conditions. Therefore, for an agrochemical product or its spray strength dilution (Howes, 1996) exposure time is recommended to be a 6-8 h period.
The exposure period is terminated by washing of the skin surface. The procedure to remove the test preparation from the surface of the skin should mimic normal practice in use. During and after cessation of exposure, sampling should be frequent and long enough in order to get insight into the absorption kinetics, which is important for derivation of the maximal flux.
For calculation of dermal absorption on basis of *in vitro* studies see chapter 4 (sections 4.2 and 4.4).

3.2 In vivo studies

3.2.1 Animal studies
The test should be carried out in accordance with "OECD guideline for the testing of chemicals, draft new guideline 427. Skin absorption: *in vivo* method" (OECD, 2000b) and the Draft OECD Guidance Document for the Conduct of Skin Absorption Studies (OECD, 2000c).
In the case of agrochemicals the exposure period should reflect the occupational exposure in use. Therefore, for an agrochemical product or its spray dilution (Howes, 1996) an exposure period of 6-8 h is considered appropriate. The exposure period is terminated by washing of the skin surface. The procedure to remove the test preparation from the surface of the skin should mimic normal practice in use. In order to get insight in the faith of the amount located in the skin, the sampling time should be long enough, e.g. until serial non-detects in excreta. For calculation of dermal absorption on basis of in vivo studies see chapter 4 (section 4.3).

3.2.2.Human volunteer studies
In agreement with the SCPH, no human volunteer studies would be done until the Ethic Committee gives its opinion about it, (amendment to the present document will be released in due time).
When dermal absorption data from human volunteer studies using existing active substance are already available, outcomes of these studies can be used for dermal absorption evaluation.
Results from field studies, if well conducted, and especially biomonitoring data may be helpful to confirm results obtained from experimental dermal in vivo and in vitro testing.

4 Decision making process for setting dermal absorption percentages

4.1 Dermal absorption based on default values

Although, in general, correlations between commonly available physical and chemical properties and dermal absorption seem to be poor (Durkin, 1995), based on theoretical considerations on skin permeation, it might be expected that there should be an optimum in log P_{ow} and a maximum in MW for facilitating percutaneous absorption. Unfortunately, clear cut-off values for negligible, low and/or high dermal absorption of chemicals cannot be derived from data presented in literature. The following criteria were proposed by De Heer (1999) to discriminate between chemicals with high and low dermal absorption:

- 10% dermal absorption is used in case MW > 500 and log P_{ow} is smaller than -1 or higher than 4, otherwise
- 100% dermal absorption is used.

The lower limit of 10% was chosen, because the data presented in literature indicate the occurrence of dermal absorption for tested compounds even beyond the extremes of log P_{ow} and/or MW values. It is noted that, by expert judgement, a deviation from 100% and 10% dermal absorption can be chosen, on a case by case basis taking into account all data available (e.g. data on water solubility, ionogenic state, 'molecular volume', oral absorption and dermal area dose in exposure situations in practice).

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

An estimate of dermal absorption can not be deduced from the results of acute toxicity studies because of the fact that differences in e.g. oral and dermal LD_{50} values are not necessarily a result of differences in absorption. First, the result in a dermal LD_{50} study is dependent on the size of the exposed area and can be changed by altering the exposed area. Second, differences in toxicity after oral and dermal exposure could be the result of first-pass effects (i.e. substance is (in)activated in the liver). Furthermore, the toxicity of a substance is also influenced by the rate of absorption. Generally, and especially in acute (gavage) studies, oral absorption will be relatively fast, resulting in a peak concentration in the body, whereas the absorption after dermal exposure is generally more gradual. Finally, for setting LD_{50} values usually high levels of test compound are given. Since absorption percentages are highly dependent on the applied dose, this may very well lead to underestimation of absorption percentages at (low) occupational exposure levels. Based on these considerations, it can be concluded that the results of acute toxicity studies can only be used to indicate high, but not a low, dermal absorption.

The use of mathematical skin permeation models for quantitative risk assessment purposes is limited because these models have generally been validated by *in vitro* data ignoring the fate of the skin residue levels. However, these models may prove useful as a screening tool or for qualitative comparison of skin permeation potential.

4.2 Dermal absorption based on *in vitro* human and rat studies

There is an increase in the number of *in vitro* studies being submitted for registration purposes. For quite a number of compounds *in vitro* methods (Bronaugh, 1991a ; Scott, 1992 ; Fed. Reg., 1996, OECD, 2000a) have demonstrated to provide a good prediction of *in vivo* dermal absorption (Franz, 1975 ; Bronaugh, 1982 ; Scott, 1987, 1992 ; Hotchkiss, 1992 ; ECETOC, 1993 ; Ramsey, 1994). It should be realized, however, there is still a debate going on how *in vitro* data could or should be used in risk assessment. Recently, an evaluation of available data on *in vitro* dermal absorption was performed under auspices of the OECD (OECD, 2000d). Because the available studies, comparing *in vitro* and *in vivo* test results, contained too many variables (different species, thickness and types of the skin, exposure duration, vehicles, etc.), evaluation of

in vitro test methods by means of data available from public literature appeared to be difficult (OECD, 2000d). A major issue of concern in the *in vitro* procedure turned out to be the presence of test substance in the various skin layers, i.e., absorbed into the skin but not passed into the receptor fluid. It was noted that it is especially difficult to examine very lipophilic substances *in vitro*, because of their low solubility in most receptor fluids. By including the amount retained in the skin *in vitro*, a more acceptable estimation of skin absorption can be obtained. Water-soluble substances can be tested more accurately *in vitro* because they more readily diffuse into the receptor fluid (OECD, 2000c and 2000d). At present, provided that skin levels are included as absorbed, results from *in vitro* methods seem to adequately reflect those from *in vivo* experiments supporting their use as a replacement test to measure percutaneous absorption (see Figure 1). This calculation, i.e. the inclusion of the amount located in the skin as being absorbed, may result in conservative estimate of the amount becoming systemically available in vivo. If refinement is needed, it should be convincingly demonstrated that the skin dose does not become absorbed at a later stage.

The maximum flux at relevant exposure levels (in $mg/cm^2/h$; calculated from the linear part of the absorption vs. time curve) derived from *in vitro* studies can be used for semi-quantitative comparison of absorption of chemicals between species, between compounds within one species, and between different vehicles within one species (provided they are tested under otherwise identical and relevant test conditions).

In case studies are not tuned into the anticipated exposure situation, e.g. with regard to type of formulant or concentration, scientific argumentation should be provided before such data can be used with confidence.

Preferably human in vitro dermal absorption studies should be carried out. When only rat in vitro dermal absorption studies are available, the most conservative approach would be to assume that human skin absorption would be equal to rat dermal absorption.

4.3 Dermal absorption based on *in vivo* data

For the conduct of *in vivo* dermal absorption studies see section 3.2 and OECD 428 (OECD 2000b). The calculation of the percentage dermal absorption from *in vivo* studies is dependent on the sampling time. If sampling is done over a sufficiently long period of time (e.g. until serial non-detects in excreta), the amount detected in the application site after washing should not be included in the amount absorbed. In this case, absorption is defined as the total amount excreted in urine, faeces and air and the amount recovered in tissue and carcass. In case excretion of the substance and/or its metabolites has not come to an end within the sampling period, but there are indications of a clear decrease in excretion, only a part of the skin bound dose may be included in the absorption by expert judgement (Thongsinthusak, 1999 ; De Heer, 1999). In case the experiment is terminated before serial non-detects in excreta are observed and/or no clear decline in excreta is measured, the amount located in the skin should be considered as being absorbed (Chu, 1996) (see Figure 1).
As applies to *in vitro* studies, experimental dermal absorption percentages used in risk assessment should be determined on in use preparation. Scientifically based arguments and expert judgement is required when dermal absorption percentage is determined on other preparation or on the active substance alone.

When only rat in vivo dermal absorption studies are available, the most conservative approach would be to assume that human skin absorption would be equal to rat in vivo dermal absorption.

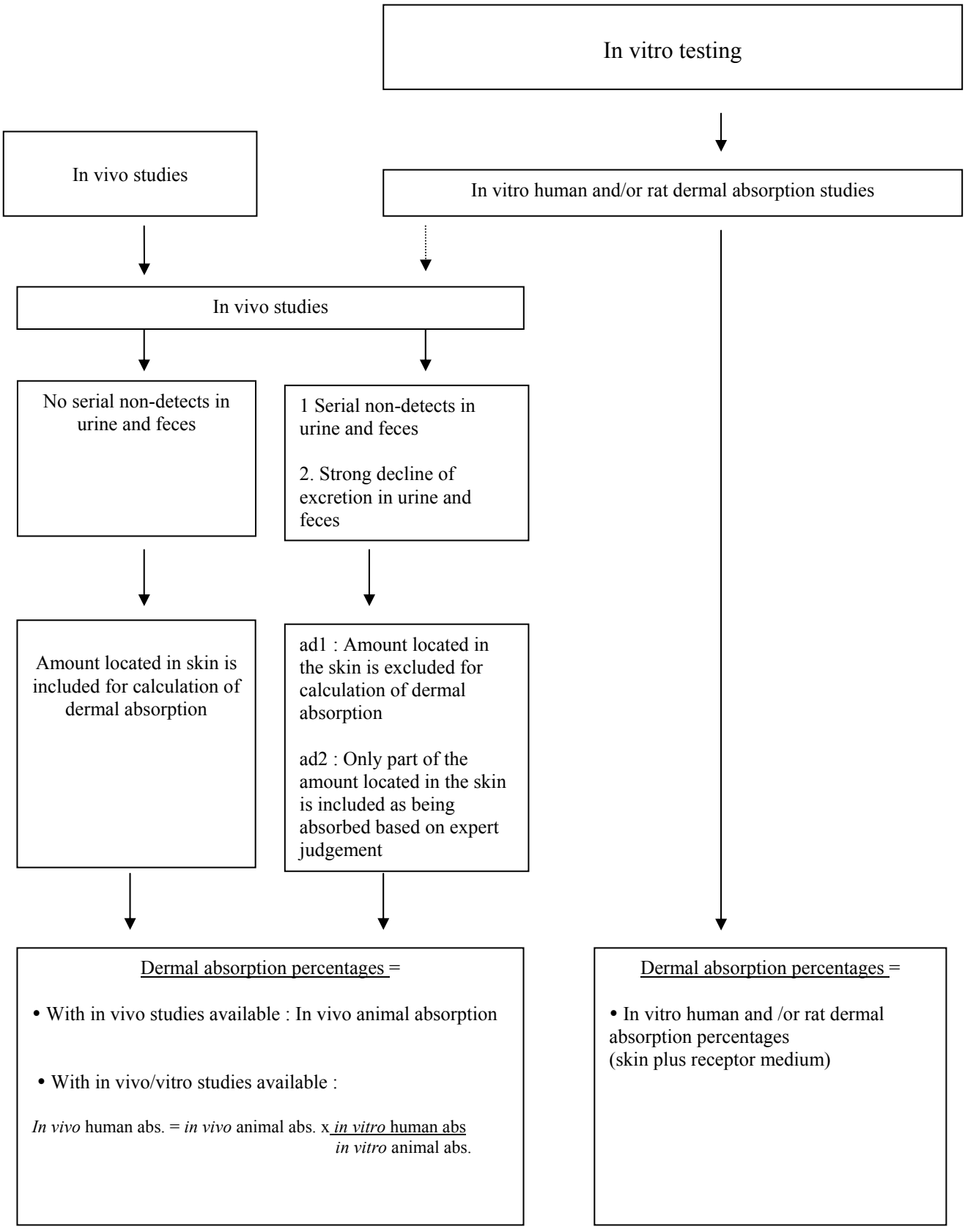
4.4 Dermal absorption percentage based on in vivo rat studies in combination with in vitro data

If appropriate dermal penetration data are available for rats *in vivo* and for rat and human skin *in vitro*, the *in vivo* dermal absorption in rats may be adjusted in light of the relative absorption through rat and human skin *in vitro* (see equation 1 and Figure 1). The latter adjustment may be done because the permeability of human skin is often lower than that of animal skin (e.g., Howes, 1996). A generally applicable correction factor for extrapolation to man can however not be derived, because the extent of overestimation appears to be dose, substance, and animal specific (Bronaugh and Maibach, 1987; ECETOC, 1993). For the correction factor based on in vitro data, preferably maximum flux values should be used. Alternatively, the dermal absorption percentage (receptor medium plus skin dose) may be used. Because, by definition, the permeation constant (Kp in cm/hr) is established at infinite dose levels, the usefulness of the Kp for dermal risk assessment is limited.

Eq. 1 $$\textit{In vivo}\ \text{human absorption} = \textit{in vivo}\ \text{animal absorption} \times \frac{\textit{in vitro}\ \text{human absorption}}{\textit{in vitro}\ \text{animal absorption}}$$

Similar adjustments can be made for differences between formulants (e.g. in vivo active substance in rat and in vitro rat data on formulants and active substance)

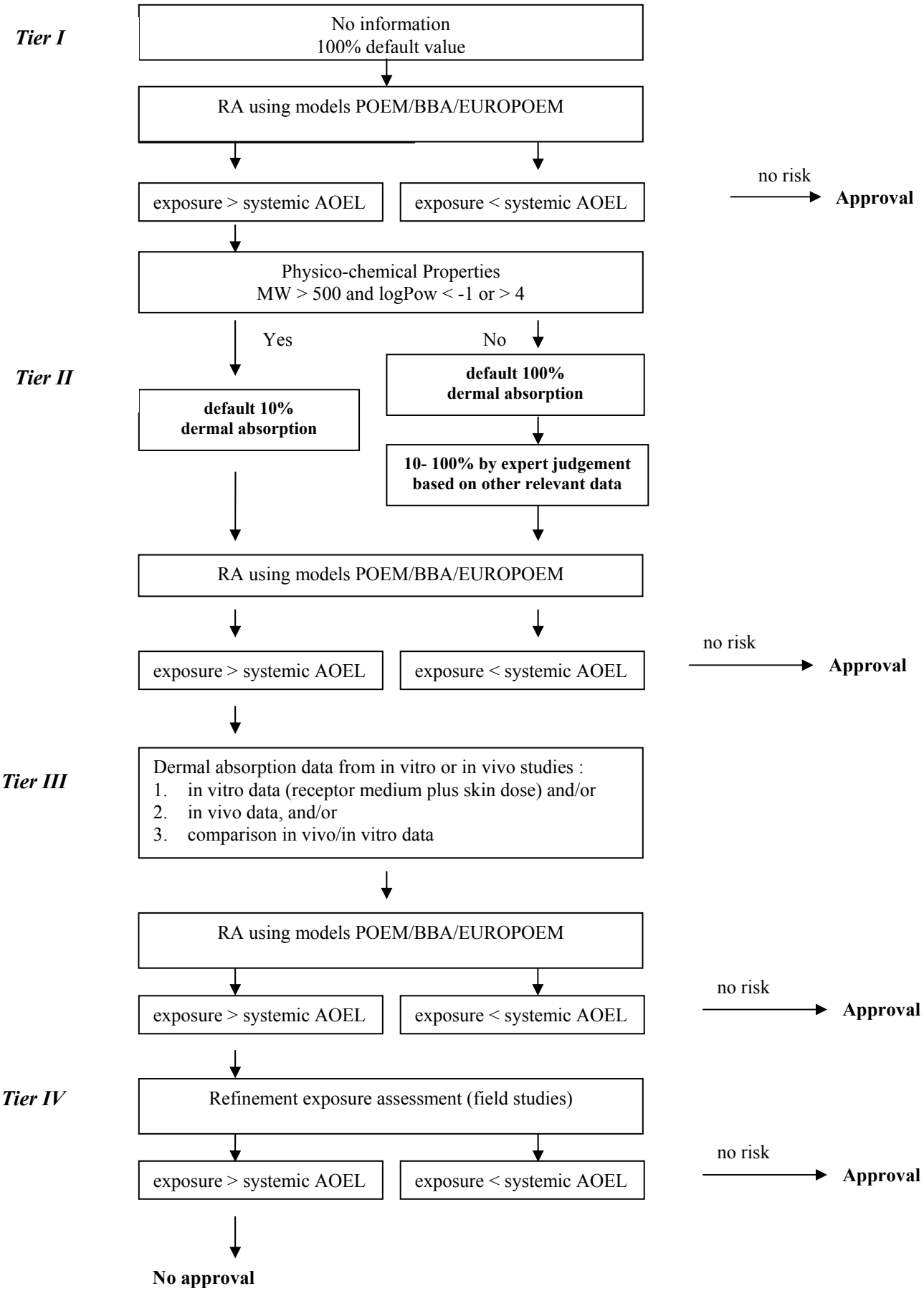
Figure 1 : Overview of the possible use of in vitro and in vivo data for setting the dermal absorption percentage.



5 Proposal for a tiered approach to risk assessment for operator exposure, using default dermal absorption percentage or dermal absorption percentage determined experimentally

The establishment of a value for dermal absorption may be performed by use of a tiered approach from a worst case to a more refined estimate (De Heer, 1999) (see figure 2). If an initial assessment ends up with a risk, more refinement could be obtained in the next tier if more information is provided on the dermal absorption. In a *first tier* of risk assessment, a worst case value for dermal absorption of 100% could be used for external dermal exposure in case no relevant information is available (Benford, 1999). An estimate of dermal absorption could be made by considering other relevant data on the substance (e.g., molecular weight (MW), log P_{ow} and oral absorption data) (*second tier*) or by considering experimental in vitro and in vivo dermal absorption data (*third tier*, see section 4). If at the end of the third tier still a risk is calculated, the risk assessment could be refined by means of actual exposure data (*fourth tier*) (Figure 2). This approach provides a tool for risk assessment, and in general it errs on the safe side.

Figure 2: Dermal absorption in risk assessment for operator exposure; a tiered approach.



Literature references

Bartek M.J., Labudde J.A., and Maibach H.I., (1972). Skin permeability in vivo: Comparison in rat, rabbit, pig and man. J Invest. Dermatol. 58: 114-123.

Benford D.J., Cocker J., Sartorelli P., Schneider T., Van Hemmen J.J. and Firth J.G., (1999). Dermal route in systemic exposure. Scand. J. Work Environ. Health 25: 511-520.

Brandau et Lippol, (1982). Dermal and Transdermal absorption, Wissenschaftliche Verlag Gesellschaft, 257 p., Stuggart, 1982.

Bronaugh R.L., and Franz T.J., (1986). Vehicle effects on percutaneous absorption: *in vivo* and *in vitro* comparisons with human skin. Br. J. Dermatol. 115: 1-11.

Bronaugh R.L., Stewart R.F., Congdon E.F., and Giles, Al. Jr. (1982). Methods for in vitro percutaneous absorption studies. I. Comparison with in vivo results. Toxicol. Appl. Pharmacol. 62: 474-480.

Bronaugh R.L. and Maibach H.I., (1987). *In vitro* percutaneous absorption. In: Dermatotoxicology, Eds. F.N. Marzulli, H.I. Maibach (3rd ed. Hemisphere Publishing, Washington DC, USA, 1987, pp.121-134).

Bronaugh R.L., Wester R.C., Bucks D., Maibach H.I., and Sarason R., (1990). In vivo percutaneous absorption of fragrance ingredients in rhesus monkeys and humans. Fd. Chem. Toxic. 28: 369-373.

Bronaugh R.L., and Collier, S.W., (1991a). Protocol for *In vitro* Percutaneous Absorption Studies, in In vitro Percutaneous Absorption: Principles, Fundamentals and Application*s*, R .L. Bronaugh and H. I. Maibach, Eds., CRC Press, Boca Raton, 1991, pp.237-241.

Bronaugh R.L., and Maibach H.I., (1991b). In vitro percutaneous absorption: principles, fundamentals, and applications. CRC Press, Boca Raton.

Chu I., Dick D., Bronaugh R., Tryphonas L., (1996). Skin reservoir formation and bioavailibility of dermally administered chemicals in hairless Guinea pigs. Fd. Chem. Toxic. 34: 267-276.

De Heer C., Wilschut A., Stevenson H., Hackkert B.C., (1999). Guidance document on the estimation of dermal absorption according to a tiered approach : an update. TNO Report V98 1237, 27p, January 1999, Zeist, The Netherlands.

Diembeck W., Beck H., Benech-Kiefer F., Courtellemont P., Dupuis J., Lovell W., Paye M., Sprengler J. and Steiling W., (1999). Test guidelines for *in vitro* assessment of dermal absorption and percutaneous penetration

Durkin P.R., Rubin L., Withey J. and Meylan W., (1995). Methods of assessing dermal absorption with emphasis on uptake from contaminated vegetation. Toxicol. Ind. Health 11: 63-79.

EC Draft guidance document, Guidance for the setting of an acceptable operator exposure level (AOEL), 7531/VI/95 rev.5, 03/01/2001. European Commission, Health and consumer protection Directorate General.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), (1993). Percutaneous absorption. Monograph n° 20, ECETOC, Brussels, Belgium.

EPA (United States Environmental Protection Agency) (1992) Dermal exposure assessment: principles and applications. Interim report EPA/600/8-91.001B. Office of Research and Development, Environmental Protection Agency, Washington D.C., USA.

EPA (United States Environmental Protection Agency) (1996) Health Effect Test Guidelines. Dermal Penetration (EPA 712-C-96-350).

EPA (United States Environmental Protection Agency) (1999) Proposed rule for *in vitro* dermal absorption rate testing of certain chemicals of interest to occupational safety and health administration. Federal Register, Volume 64, Number 110, June 9, 1999.

Feldman R.J., and Maibach H.I., (1970). Absorption of some organic compounds through the skin in man. J. Invest. Dermatol. 54, 399-404.

Feldman R.J., Maibach H.I., (1974). Percutaneous penetration of some pesticides and herbicides in man. Toxicol. Appl. Pharmacol., 28 :126-132.

Flynn GL (1985) Mechanism of percutaneous absorption from physicochemical evidence. In: *Percutaneous absorption; mechanisms-methodology-drug delivery*, Bronough R and Maibach H.I. (Eds), Marcel Dekker; pp. 17-42, New York.

Franz T.J., (1975). Percutaneous absorption: on the relevance of in vitro data. J. Invest. Dermatol. 64: 190-195.

Recommended Protocol for In vitro Percutaneous Absorption Rate Studies (1996). Federal Register, Vol 61, No. 65.

Hotchkiss S.A.M., Hewitt P., Caldwell J., Chen W.L. and Rowe R.R., (1992). Percutaneous absorption of nicotinic acid, phenol, benzoic acid and triclopyr butoxyethyl ester through rat and human skin in vitro: Further validation of an *in vitro* model by comparison with *in vivo* data. Fd. Chem. Toxic. 30: 891-899.

Howes D., Guy R., Hadgraft J., Heylings J.R., Hoeck U., Kemper F., Maibach H., Marty J.P., Merk H., Parra J., Rekkas D., Rondelli I., Schaefer H., Taüber U., Verbiese N., (1996). Methods for assessing percutaneous absorption. ECVAM Workshop Report ATLA 24 81.

Kemppainen B.W., and Reifenrath W.G., (1990). Methods for skin absorption. CRC Press, Boca Raton.

Lee C.L., Uchida T., Kitagawa K., Yagi A., Kim N.-S., and Goto S., (1994). Relationship between lipophilicity and skin permeability of various drugs from an ethanol/water/lauric acid system. Biol. Pharm. Bull. 17: 1421-1424.

Maibach H.I., Wester R.C., (1989). Percutaneous absorption: In vivo methods in humans and animals. J. Am. Coll. Tox. 8: 803-813.

Noonan P.K. and Wester R.C. (1989). Cutaneous metabolism of xenobiotics. In: Bronough RL, Maibach HI (Eds) Percutaneous absorption. New York: Marcel Dekker; pp 53-75.

OECD, (2000,b). OECD Guideline for the Testing of Chemicals. Draft Guideline 427: Skin Absorption *in vivo* method (December 2000)

OECD, (2000a). OECD Guideline for the Testing of Chemicals. Draft Guideline 428: Skin absorption*: in vitro* method (December 2000).

OECD, (2000c). Draft guidance document for the conduct of skin absorption studies. OECD environmental Health and Safety Publications Series on Testing and Assessment No 28. (December 2000).

OECD (2000d) Test Guidelines Program. Percutaneous absorption testing: is there a way to consensus?, OECD document ENV/JM/TG(2000)5, April 2000, Paris France

Ramsey J., Woollen B.H., Auton T.R. and Scott R.C. (1994). The predictive accuracy of *in vitro* measurements for the dermal absorption of a lipophilic penetrant (fluazifop-butyl) through rat and human skin. Fundam. Appl. Toxicol. 23: 230-236.

Ross et al., (1992). Experimental method to estimate indoor pesticide exposure to children. In Similarities and Differencies Between Children and Adults : Implications for risk assessment (P.S. Guzelian, C.J. Henry and S.S. Olin Eds.), 226-241. ILSI Press, Washington, DC.

Ross J.H., Dong M.H. and Krieger, R.I. (2000). Conservatism in pesticide exposure assessment. Reg. Toxicol. Pharmacol. 31: 53-58.

Schaefer H., and Redelmeier T.E., (1996). Skin barrier: principles of percutaneous absorption. Karger, Basel.

Scott R.C., and Ramsey J.D., (1987). Comparison of the *in vivo* and *in vitro* percutaneous absorption of a lipophilic molecule (Cypermethrin, a pyrethroid insecticide). J. Invest. Dermatol. 89: 142-146.

Scott R.C., Corrigan M.A., Smith F., Mason H., (1991). The influence of skin structure on permeability: an intersite and interspecies comparison with hydrophilic penetrants. J. Invest. Dermatol. 96: 921-925.

Scott R.C., Batten P.L., Clowes H.M., Jones B.K. and Ramsey J.D., (1992). Further validation of an *in vitro* method to reduce the need for *in vivo* studies for measuring the absorption of chemicals through rat skin. Fundam. Appl. Toxicol. 19: 484-492.

Scott R.C., Carmichael N.G., Huckles K.R., Needham D., and Savage T., (1993). Methods for measuring dermal penetration of pesticides. Fd. Chem. Toxic 31: 523-529.

Southwell D., and Barry B.W., (1983). Penetration enhancers for human skin: mode of action of 2-pyrolidone and dimethylformamide on partition and diffusion of model compounds water, n-alcohols and caffeine. J. Invest. Dermatol 80: 507-514.

Thongsinthusak T., Ross J.H., Saiz S.G., Krieger R.I., (1999). Estimation of Dermal absorption Using the Exponential Saturation Model, Reg. Toxicol. Pharmacol.29: 37-43.

Van de Sandt J.J.M., Meuling W.J.A., Elliott G.R., Cnubben N.H.P. and Hakkert B.C., (2000). Comparative *in vitro* - *in vivo* percutaneous absorption of the pesticide propoxur. Toxicological Sciences 58: 15-22.

Wépierre J., (1970). L'absorption percutanée, Produits pharmaceutiques, vol.26, n°6, 312-329, 1970.

Wolfe H.R., (1976). Field exposure to airborne pesticides (chapter 5). In Air Pollution from Pesticides and Agricultural processes (R.E. Lee, Jr., Ed.), 137-161. CRC Press, Ohio.