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Comparison of the human skin grafted onto nude mouse model with *in vivo* and *in vitro* models in the prediction of percutaneous penetration of three lipophilic pesticides

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Abstract

The evaluation of the degree of percutaneous penetration of agrochemicals is a key part of risk assessment for operators. The availability of suitable and predictive experimental models is crucial, in particular in the case of lipophilic compounds which persist in the stratum corneum (SC). Regulatory models (rat *in vivo*, human and rat *in vitro*) and the innovative human skin grafted onto nude mice (HuSki) model were compared for their ability to predict the human skin absorption. Radiolabelled malathion, lindane and cypermethrin (4 μg/cm^2) were topically applied to each model. The % of applied dose absorbed and that present in skin and SC were evaluated at 24 h. Additionally, the absorption profile of cypermethrin was evaluated in the *in vivo* rat and HuSki models for up to 11 days. We found that the human *in vitro* and HuSki models closely predicted the human skin absorption at 24 h, while rat models overestimated the human skin absorption. Furthermore, our experiments with cypermethrin indicated that evaluation of % percutaneous absorption over extended periods of time was feasible with the HuSki model. In our studies the HuSki model overcame the limitations of the regulatory models and is promising to realistically refine the dermal absorption assessment of topically applied chemicals.

Keywords: Dermal penetration/absorption; Lindane; Cypermethrin; Malathion; Human skin grafted onto nude mouse; HuSki; *in vivo*/*in vitro*; Rat/human; Comparative study; Stratum corneum

1. Introduction

The skin is a natural barrier that normally prevents significant systemic exposure to environmental chemicals. The primary barrier function of the skin is provided by the stratum corneum (SC),[4] the most external layer of the skin (Elias and Menon, 1991); (Holleran et al., 1991). This layer is made up of multiple layers of flattened keratinocytes, held together with a continuous lipid layer. The structure of the SC is sometimes described as being like the "bricks and mortar of a wall"; the hydrophilic cells with the hydrophobic lipid "mortar" layer makes up a strong, highly flexible, self-repairing barrier (Elias, 2005).

The penetration of chemicals through the SC is a passive process (Elias et al., 1987) and is highly dependent on the physicochemistry of individual chemicals, particularly on the lipophilicity of the compound, and on the vehicle in which the chemical is applied. The penetration of chemicals

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[4] *Abbreviations used:* HuSki, human skin grafted on nude mice; PP, percutaneous penetration; SC, stratum corneum; RF, receptor fluid; OECD, Organisation for Economic Cooperation and Development; AOEL, Acceptable operator exposure level; MBq, megabecquerel; BSA, bovine serum albumin; PBS, phosphate buffer saline; TEWL, trans-epidermal water loss; LSC, liquid scintillation counting.

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also depends on the species-dependent skin characteristics. In particular, the morphology and biochemistry of the SC is different in rodent skin and in human skin (Bartek et al., 1972; Bronaugh et al., 1982). In rodents, the SC is thin (about 5–15 μm, and around to 2–3 layers of corneocytes) and the principle protection from the external environment is the fur, whereas in humans, the SC is thicker (about 10–25 μm, and around 4–15 layers of corneocytes, and even more in the most thick body areas) and constitutes the sole barrier. The hair follicles, sebaceous glands and sweat ducts (appendages) can increase the percutaneous penetration of a compound. Indeed, percutaneous penetration may occur in three ways: intercellular way, trans-cellular way, or via the appendages (short circuit way, diffusion of the molecule that can reach directly the dermis) (Lauer et al., 1995). These species differences result in different chemical penetration characteristics, with rodent skin being generally more permeable than human skin to chemicals.

The skin is obviously a primary route of exposure for agrochemical users, with possible contact arising during the mixing and loading process or during field application (Benford et al., 1999). The assessment of the degree of skin penetration of chemicals is therefore a key part of risk assessment, since it is used together with exposure data to evaluate how much test chemical could be systemically available in exposed workers on a mg/kg/day basis. This value is then compared with the acceptable operator exposure level (AOEL) deduced from the toxicology data generated in animal models, to ensure safe use of the plant-protection products. Thus, to accurately assess the degree of absorption of chemicals through the skin of operators, it is necessary to have suitable predictive experimental models. The ideal model for estimating human skin penetration would involve human *in vivo* studies determining the dermal kinetics of a compound. However, technical difficulties, ethical issues, and an unwillingness of some regulatory authorities to accept human volunteer data make it impractical in most cases. At the other extreme, predictive mathematical models based only on chemical structure are widely available (Lien and Gao, 1995) and are relatively reliable for pure single chemicals in saturated aqueous solutions. However, these mathematical models are not currently appropriate for use in the risk assessment of complex mixtures such as agrochemical formulations (Surber et al., 1990; Guy and Potts, 1993).

The assessment of the skin absorption of complex mixtures often requires the use of *in vivo* experimental models (rat, pig), where formulations (usually containing radiolabelled chemical) are applied to the shaved skin of the animals for a defined period of time, excreta are collected and the distribution of the chemical is measured at study termination. Additionally, *in vitro* studies are often performed, involving the use of human and/or animal skin in diffusion cells, where the test chemical formulation is applied to the skin surface. The amount of test chemical migrating into the receptor fluid (RF) during a specified time period is defined as the systemically absorbed amount (OECD, 2004a,b). Both *in vivo* and *in vitro* experimental models are currently used in regulatory studies to aid in the prediction of human *in vivo* skin penetration of agrochemical products (US-EPA, 1998; OECD, 2004a,b,c). However, the selection of studies required and the way the data are interpreted are not currently harmonized between regulatory authorities. For example, the US-EPA does not accept *in vitro* studies in the risk assessment process. Furthermore, *in vitro* models are only validated to a limited extent with *in vivo* models, as *in vitro* data are often compared to *in vivo* data obtained from studies using different experimental conditions (in terms of dose applied, exposure duration, vehicle and species) (OECD, 2000b). To study the validity of *in vitro* and *in vivo* approaches for the prediction of the human skin absorption, it is of critical importance to directly compare *in vitro* and *in vivo* models under similar conditions. In addition, the question of whether the fraction of compound remaining in the skin and stratum corneum at the end of the experiment must be considered as systemically absorbed or not, is the subject of much debate (OECD, 2000a). This point is of particular interest for lipophilic compounds, which have a tendency to accumulate in the SC.

The main objective of this paper was to compare the skin penetration for 3 reference insecticides (malathion, lindane and cypermethrin) using two *in vivo* and two *in vitro* experimental models. Although we compare the data obtained from our models to human volunteer data (Maibach et al., 1971; Feldmann and Maibach, 1974; Woollen et al., 1992), it should be noted that the human data represent an approximation of what the real absorption value should be. Specifically, the human data represent the values obtained from urinary excretion alone collected for 5 days, but corrected by comparison with iv injection study data (this correction gives a more accurate estimation of the human skin absorption). In addition, the use of a soya-based formulation in the volunteer studies may result in lower penetration rate compared to acetone. Thus the comparison with human volunteer data is only indicative.. The experimental models used in this study were selected to represent the most common *in vivo* and *in vitro* models used in regulatory studies, namely the rat *in vivo* model and human and rat *in vitro* models. In addition, an *in vivo* model already described by Reifenrath et al., 1984 , which is more akin to the human *in vivo* situation, was evaluated and compared with the current regulatory models. This new model uses Human Skin grafted onto nude mice (the "HuSki" model), and therefore has the advantage of allowing the evaluation of chemicals using a system consisting of a viable human skin and SC with a physiological capillary circulation. The latter point is critical, as it provides "sink conditions" equivalent in part to human *in vivo (mouse DME processes could differently modulate these sink conditions)*, the lack of which in *in vitro* models is a main issue of concern for regulatory interpretation of data.

The chemicals tested in the present study were chosen on the basis of the availability of published human skin penetration data, the availability of high purity radiola-