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New *in vitro* dermal absorption database and the prediction of dermal absorption under finite conditions for risk assessment purposes

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ABSTRACT

Most QSARs for dermal absorption predict the permeability coefficient, K_p, of a molecule, which is valid for infinite dose conditions. In practice, dermal exposure mostly occurs under finite dose conditions. Therefore, a simple model to predict finite dose dermal absorption from infinite dose data (K_p and lag time) and the stratum corneum/water partition coefficient ($K_{SC,W}$) was developed. To test the model, a series of *in vitro* dermal absorption experiments was performed under both infinite and finite dose conditions using acetic acid, benzoic acid, bis(2-ethylhexyl)phthalate, butoxyethanol, cortisone, decanol, diazinone, 2,4-dichlorophenol, ethacrynic acid, linolenic acid, octylparaben, oleic acid, propylparaben, salicylic acid and testosterone. For six substances, the predicted relative dermal absorption was not statistically different from the measured value. For all other substances, measured absorption was overpredicted by the model, but most of the overpredictions were still below the European default absorption value. In conclusion, our finite dose prediction model provides a useful and cost-effective estimate of dermal absorption, to be used in risk assessment for non-volatile substances dissolved in water at non-irritating concentrations.

1. Introduction

Dermal absorption data are needed in toxicological risk assessment of chemical substances in order to estimate internal dose after dermal exposure to these chemicals. This internal dose can then be compared to an internal limit value for systemic effects to assess the safety of the dermal exposure (see a.o. the EU technical guidance on risk assessment: EU, 2004). Dermal absorption can be estimated using *in vivo* or *in vitro* studies with humans or animals, which need to be conducted under conditions mimicking those expected to occur during the exposure(s) to be evaluated for toxicological risk (EU, 2004). When no experimental data are available, European regulatory authorities assume 100% dermal absorption unless the chemical possesses a molecular weight >500 and a log K_{ow} smaller than −1 or higher than 4, in which case 10% absorption is assumed (EU, 2004). In our experience, most molecules evaluated in regulatory risk assessment are assumed to be totally absorbed, when applying this rule.

At the end of the year 2006, the EU parliament accepted new legislation on the registration of chemicals, known under the acronym REACH.[1] REACH entered into force on 1st June 2007. Based upon it, over 50,000 existing chemicals will need to be evaluated for environmental and health safety under the conditions they are manufactured, used and discarded. All substances should be evaluated by the end of 2017. For many chemicals, data on dermal absorption under many different exposure conditions will need to be generated. To save time and money, a simple *in silico* method, not automatically leading to a default of 100% absorption, would be advantageous. Extensive overviews of available *in silico* methods have been published recently (WHO, 2006; Bouwman et al., 2008). Most QSARs calculate the permeability coefficient, K_p, of a molecule, which is a measure of the skin permeability of a molecule under steady state conditions. Steady state is reached after a certain lag time, which amongst others depends on the nature of the molecule. The K_p is usually expressed in cm/h, and from it the flux of solute over the skin under steady state conditions can be calculated for

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[1] Registration, Evaluation Authorisation and restriction of CHemicals.

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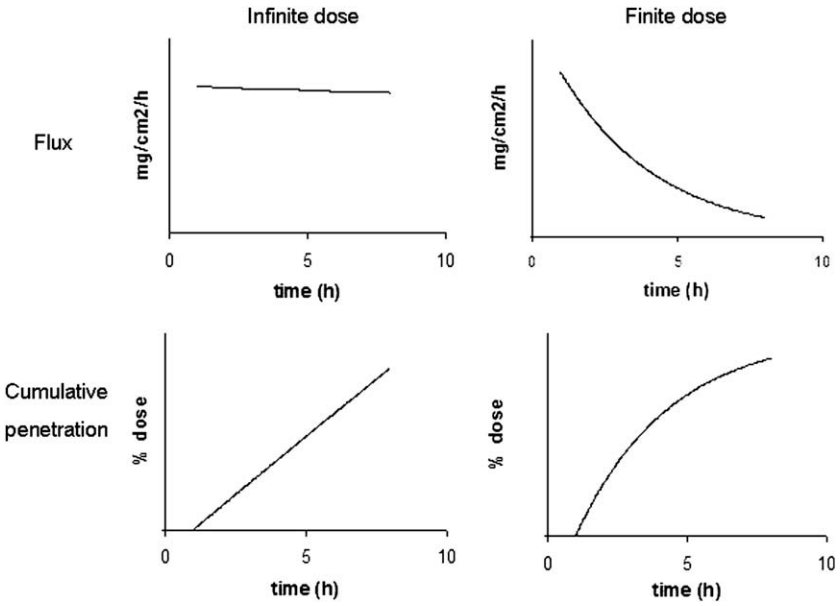

Fig. 1. Comparison of infinite and finite dose dermal penetration.

so called infinite dose conditions, i.e., when the concentration of the solution applied to the skin does not change (appreciably) over time. In practice, however, dermal exposure will mostly occur under finite in stead of infinite dose conditions, meaning the concentration of the solute in solution on the skin will clearly change over time. Under these conditions, the flux of the substance across the skin will decrease with time as the solution on the skin is depleted of its solute (see Fig. 1). Using K_p values and initial concentrations to calculate absorption would thus lead to overestimation. Therefore, we developed a simple model to predict finite dose absorption from infinite dose data (K_p and lag time). In order to test this finite dose absorption prediction model, a series of *in vitro* dermal absorption experiments was performed with 15 different substances, both under infinite and finite dose conditions.

2. Materials and methods

2.1. In vitro dermal absorption experiments

The *in vitro* skin absorption assays were executed according to standard protocols used in our laboratory to perform *in vitro* dermal absorption studies according to OECD test guideline 428 (OECD, 2004). The assays were performed in static diffusion cells using cryopreserved human abdominal skin (exposed area 0.64 cm^2), as previously described by van de Sandt et al. (1993, 2000). The skin originated from nine female donors, aged 29–53 years (average 39 ± 7.4). Epidermal membranes were used, which were prepared by incubating skin overnight in 2 M NaBr solution in saline, after which the epidermis was peeled from the dermis using forceps. The receptor fluid (total volume 1.2 mL) consisted of a physiological salt solution (0.9% NaCl w/v) containing 0.01% sodium azide and 6% polyoxyethylene (20) oleyl ether, the latter having been added to ensure also lipophilic test substances would be readily soluble in it. Prior to the start of the experiment, integrity of the epidermal membranes was assessed by determining the permeability coefficient (*Kp*) of tritiated water, as described by van de Sandt et al. (1993, 2000). Epidermal membranes with a K_p for tritiated water of less than 3.0×10^{-3} cm/h were used in the subsequent experiments.

The epidermal membranes were exposed to 780 μL (the maximum volume the donor cells of our system can accommodate) or 16 μL aqueous dose solution/cm^2, representing infinite and finite dose experiments,[2] respectively.

For the absorption experiments in which K_p was assessed over a broad range of test concentrations for seven substances, the aim was to test 5× a 1:5 dilution of the test compounds. For a number of substances, the highest test concentrations were selected based on maximum solubility in water, which was determined experimentally before preparation of the solutions (octylparaben, propylparaben, testosterone, 2,4-dichlorophenol and decanol). As the flux is determined by the concentration of the substance and its K_p, it is not useful to test beyond maximum water solubility. For the other compounds solubility was not a limiting factor. Butoxyethanol and ethylene glycol were tested at a maximum concentration of 50% (v/v) (corresponding to, respectively, ca. 470 and ca. 530 mg mL^{-1}). Acetic acid was tested at a lower maximum concentrations of 100 mg mL^{-1}, as this compound is known to have corrosive potential. According to EU classification, 10% acetic acid constitutes the lower limit for classification as "irritating". This means a series of concentrations was used up to the point where, according to EU classification, irritancy starts. In the absorption experiments it was shown that the K_p is relatively constant over a broad range of test concentrations. Therefore, test concentration selection for the other experiments was less critical. The test concentrations of the different test compounds are presented in Table 1. All concentrations chosen were below the maximum solubility of the test substance in water. All test solutions were prepared using a [^{14}C]-radiolabel. Depending on the specific activity of the radiolabel and the desired test concentration, the radiolabel was mixed with unlabelled test compound. The dose compartments were occluded using a glass coverslip fixed to the rim of the donor cell with Vaseline, in order to avoid evaporation.

In all experiments, the epidermal membranes (3–4 per test group) were exposed to the dose solutions for the whole experimental period. The duration of the finite dose experiments was set to 8 h to represent one working day. The infinite dose experiments were much longer in duration (48–51 h), in order to make

[2] For very slowly penetrating substances even 16 μL/cm^2 may constitute an infinite dose. However, as the results for our experiments show, this was not the case for our test substances.