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# Dermatokinetics of didecyldimethylammonium chloride and the influence of some commercial biocidal formulations on its dermal absorption *in vitro*

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**Abstract**

The *in vitro* dermal absorption kinetics of didecyldimethylammonium chloride (DDAC) was studied after single and multiple exposure. In addition, the influence of biocidal formulations on the absorption of DDAC was investigated. Following dermal exposure to DDAC in aqueous solution, less than 0.5% of the applied dose reached the receptor fluid after 48 h. The apparent permeability coefficient ($K_p$) was $5 \pm 1$ cm/h $\times 10^{-6}$ for concentrations <12.5 mg/mL, and $12 \pm 3$ cm/h $\times 10^{-6}$ for concentrations $\geqslant$12.5 mg/mL, suggesting that DDAC decreases the skin barrier function. DDAC distributed readily into the *stratum corneum*, but the *dermis* appeared to be the main barrier for DDAC penetration. Multiple dosing of DDAC increased its flux across the skin, when applied in high concentrations (>11 mg/mL). However, the amount of DDAC reaching the receptor fluid remained low (<1% over a 48 h period). Selected biocidal formulations tended to reduce DDAC skin absorption. The degree of reduction appeared to be correlated to the amount of aldehydes present. Based on the comparison of the distribution of DDAC in full-thickness skin and epidermal membranes, we conclude that approximately one-third of the DDAC measured in the full-thickness membranes resides in the *dermis*. As a reasonable worst case assumption, this fraction could be considered systemically available when estimating the daily systemic body burden of DDAC.


*Keywords:* Biocides; Dermal absorption; Dermatokinetics; Didecyldimethylammonium chloride; Risk assessment; Skin barrier; Repeated exposure; Single exposure; Quaternary ammonium chlorides; Vehicle effects

## 1. Introduction

The quaternary ammonium chlorides alkyldimethylbenzylammonium chloride (ADBAC) and didecyldimethylammonium chloride (DDAC) have been shown to reduce the skin barrier function *in vitro* (Buist et al., 2005). Both compounds clearly and consistently increased the skin permeability of the marker compounds [$^3$H-]water and [$^{14}$C-]propoxur after single application of relatively low concentrations. These results suggest that quaternary ammonium chlorides may also enhance their own dermal uptake. A survey of the biocidal products on the market in The Netherlands revealed that of the 714 products admitted on the market at that time, 42 contained ADBAC and 166 contained DDAC. The biocidal products that contain these quaternary ammonium chlorides are disinfectants and preservatives. Especially when used in disinfectants, the probability of workers and consumers being exposed via the dermal route is high. Relatively little is known about the systemic toxicity of quaternary ammonium chlorides, but the acute oral $LD_{50}$ of DDAC in rats (84 mg/kg bw/d, BIBRA, 1990), would lead to the classification "toxic" according to EU criteria. Systemic effects (reduced urinary excretion of potassium and chloride) were observed at single oral doses of 10–50 mg/kg bw. The $LD_{50}$ of ADBAC in rats is higher than that of DDAC: 234–525 mg/kg bw, and an oral administration of 250 mg/kg bw led to

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severe congestion of liver and kidneys (Xue et al., 2004). According to EU criteria ADBAC would be classified as 'harmful'. In view of its frequent use as a disinfectant, its potential systemic toxicity and because of its effects on skin permeability, the absorption kinetics of DDAC was studied after multiple and single exposure. We used an *in vitro* model to conduct our research, as it allowed us to conduct many more experiments than would have been the case with an *in vivo* model or, even more so, with human volunteers. Properly conducted *in vitro* measurements can be used to predict *in vivo* absorption (see a.o. the recent WHO monograph on dermal absorption (WHO, 2006) and the evaluation by Hakkert et al. (2005)). In order to determine the permeability coefficient ($K_p$) of DDAC, the initial experiments were performed using a high volume of exposure (313 μL/cm$^2$) in order to approach the infinite dose conditions. Further experiments were executed using a low volume of exposure (10 μL/cm$^2$), which is more representative of normal worker and consumer exposure (finite dose). As absorption may be considerably influenced by the nature of the vehicle (Hakkert et al., 2005), the influence of various commercial biocidal formulations on DDAC absorption was investigated as well.

## 2. Materials and methods

### 2.1. In vitro experiments

The *in vitro* skin penetration assays were performed in static diffusion cells using cryopreserved human abdominal skin, as previously described by van de Sandt et al. (1993, 2000). The skin originated from four female donors, aged 28–60 years (average 43). The dermis was partly removed using forceps and scissors, attaining an average skin thickness of 0.565 ± 0.064 mm. Most experiments were executed with these full-thickness skin preparations. 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 3% BSA. Some experiments were executed using epidermal membranes in stead of full-thickness skin. They were prepared by incubating skin overnight in a 2 M NaBr solution in saline, after which the epidermis was peeled off the dermis using forceps.

In the high volume experiments, the skin preparations were exposed to 313 μL/cm$^2$ of DDAC in 7.4% 2-propanol in water for 48 h at concentrations of 0.5, 2.5, 12.5 and 50 mg DDAC/mL. In the low volume experiments, the skin preparations were exposed to 10 μL/cm$^2$ of DDAC in 7.4% 2-propanol in water or formulation for 4 h (single exposure) or for three times 4 h (repeated exposure, starting at 0, 24 and 48 h).The DDAC concentrations applied were 50 mg/mL (Roloxid 50 formulation), 27.5 mg/mL (Bakta Steril) and 11 mg/mL (MS Macrodes), respectively. These formulations were compared to equivalent solutions of DDAC in 7.4% 2-propanol in water. The experimental designs are illustrated in Fig. 1.

Each exposure period was finished by washing off the test substance using four cotton swabs humidified with a 3% Teepol solution and subsequently drying the skin preparations using two dry cotton swabs. In the repeated as well as in the single low volume experiments all skin preparations were washed three times, in order to control for a possible effect of the washing procedure on skin permeability. Each experiment was performed with skin from one donor. Most experiments were performed each with skin from a different donor.

Prior to the start of the experiment, integrity of the skin preparations was assessed by determining the permeability coefficient ($K_p$) of tritiated water, as described by van de Sandt et al. (1993, 2000). Only skin preparations with a $K_p$ of less than $3.0 \times 10^{-3}$ cm/h for tritiated water were used in the subsequent experiments.

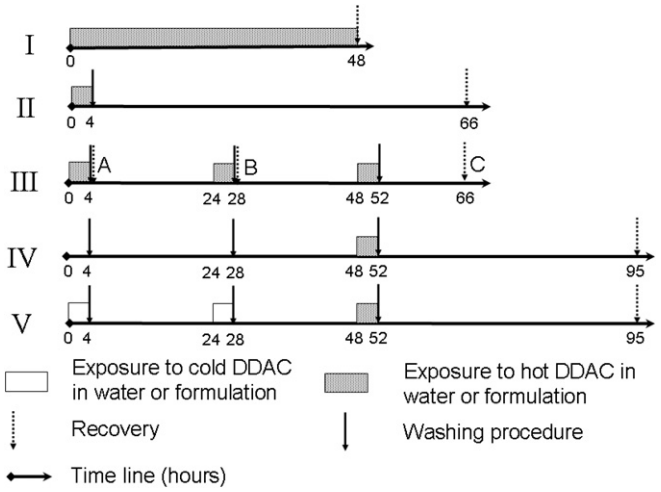


Fig. 1. Experimental design. (I) High volume exposure full-thickness skin. (II) Single low volume exposure full-thickness skin and epidermal membrane. (III) Repeated low volume exposure full-thickness skin. (IV) Single low volume exposure DDAC in water or formulation (full-thickness skin). (V) Repeated low volume exposure DDAC in water or formulation (full-thickness skin).

After the start of (the first) exposure, 500 μL samples of receptor fluid were collected at regular intervals and assayed for $^{14}$C-radioactivity by liquid scintillation counting in a Wallac Pharmacia scintillation counter. Directly after each sampling the original volume of the receptor fluid was restored by adding 500 μL fresh receptor fluid to each well. The amount of [$^{14}$C]-DDAC that had penetrated the skin was plotted against time and the penetration rate was calculated by linear regression analysis. At the end of each experiment, the recovery was determined by measurement of radioactivity in the cotton swabs fraction (cotton swabs were extracted with ethanol and a subfraction was counted for radioactivity) and the skin membrane fraction (skin membranes were dissolved in 1.5 M KOH in 20% ethanol and a subfraction was counted for radioactivity). In some experiments the skin preparations were tape-stripped seven times using D-squame (Monoderm, Monaco). Each first tape strip was collected and counted separately. Subsequent tape strips were pooled in groups of three. Radioactivity in the tape strip fractions was determined by direct addition of scintillation fluid.

[$^{14}$C]-DDAC (labelled at the methyl-groups, s.a. 2.15 GBq/mmol, radiochemical purity >99%) was purchased from Amersham, England. Unlabelled DDAC (50% solution in 2-propanol/water (2:3)) was purchased from Merck KgaA, Darmstadt, Germany. The commercially available biocides Bakta Steril, Roloxid 50 and MS Macrodes were obtained from Fisher Emergo B.V. Landsmeer, Netherlands.

### 2.2. Statistics

Statistical calculations were executed using MS Excel version 2003. First equality of variances was tested using the FTEST function to calculate the $F'$ (folded) statistic. If the two-tailed probability of a greater $F'$-value was >0.05, equality of variances was assumed, and the null hypothesis of no differences between groups was tested using the unpaired homoschedastic TTEST function. If the probability was ⩽0.05, inequality of variances was assumed, and the null hypothesis was tested using the heteroschedastic TTEST function. As the groups were relatively small (usually $n = 4$), normal distribution was assumed and not further tested.

## 3. Results

The high volume experiments (design I, Fig. 1) with DDAC showed that the $K_p$-value increased with increasing concentration (see Fig. 2). Cumulative penetration of DDAC showed almost linear kinetics between 28 and 48 h after application for the two lower concentrations, while