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Evaluation of the effect of skin cleaning procedures on the dermal absorption of chemicals

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ABSTRACT

To reduce the internal exposure, skin decontamination is the most important measure after dermal contact to chemicals. However, no harmonized skin cleaning procedure for experimental *ex vivo* studies is published. In our study, the impact of two skin cleaning techniques on dermal penetration kinetics and intradermal deposition of 1,4-dioxane, 5% hydrofluoric acid (HF, detected in terms of fluoride ions), and anisole was evaluated to develop a reliable *ex vivo* skin cleaning method using the diffusion cell technique. After exposure (duration: 3 min (HF); 1 h (1,4-dioxane and anisole)) of excised human skin ($n = 6–8$) decontamination was performed by (I) water–soaked cotton swabs or (II) direct application of water on the exposure area. The effect of skin cleaning was investigated by analysing the concentration time course of chemicals in the receptor fluid of diffusion cells and by determining the deposition in skin. Both skin cleaning procedures reduced the amount of fluoride in the skin compartments ($p < 0.05$) and the receptor fluid ($p < 0.1$). However, the effect of cleaning on the dermal absorption of the organic test compounds was not significant. The results demonstrate the suitability of the applied *ex vivo* protocol for investigating the effectiveness of skin cleaning measures following dermal exposure. In addition, data reveal that the determination of test compounds in both, skin compartments as well as receptor fluid as equivalent for the systemic uptake needs to be considered in studies assessing the effectiveness of skin decontamination procedures.

1. Introduction

Dermal exposure to hazardous substances is not always avoidable and may be a danger for health. Serious incidents after dermal contact with potentially hazardous substances at the workplace are described (Horch et al., 1994; Gear et al., 2001; Dalamaga et al., 2008). Failure in effective protection of the skin from hazardous substances even with adequate protective equipment requires an appropriate cleaning of the skin. In chemical safety data sheets, the use of neat water or aqueous solutions containing detergents are often recommended to decontaminate the skin after occupational exposure to chemicals. However, there is no proof of evidence for the effectivity of these recommendations. Thus, before employing skin cleaning techniques at the workplace, an assessment of its effectiveness is required.

Since decades, the decontamination of skin is an issue of scientific research. Latest approaches base on an innovative system for the standardisation of skin decontamination in volunteers (Sonsmann et al., 2014). However for many hazardous substances only animal or *ex vivo* studies e.g., diffusion cell studies are applicable to evaluate the effectiveness of skin cleaning. Matar et al. (2014) introduced an *ex vivo* diffusion cell design, reproducing hydrodynamic conditions for decontaminating pig skin but up to now, the system was not applied for the assessment of percutaneous penetration data of model substances using human skin. As a result of the considerable higher density of hair follicles in animals (up to 26-fold higher in rat) compared to human skin (Bronaugh et al., 1982) skin cleaning studies in animals could

Abbreviations: CAS, chemical abstract service; CV, relative coefficient of variation; DEET, N,N-diethyl-m-toluamide; FID, flame ionisation detector; HF, hydrofluoric acid; KOH, potassium hydroxide; LC–ICP–MS, liquid chromatography linked with inductively coupled plasma–mass spectrometry; LogP, decadic logarithm of the octanol/water partition coefficient; LOQ, limit of quantification; *m/z*, mass-to-charge ratio; NaCl, sodium chloride; RT, room temperature; SD, standard deviation; SEM, standard error of the mean.

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overestimate the human exposure due to the larger follicular reservoir and a consequently increased internal uptake of chemicals. Therefore, dermal absorption data from animal studies are often not readily transferable to human beings (Scott et al., 1986; Kenyon et al., 2004; Williams, 2006).

Several studies on skin decontamination are available, varying in species (mouse, pig, human), cleaning agents (e.g. solvents (Hughes et al., 2001), water (Zhai et al., 2008), soap and water (Nielsen, 2010)), and duration or frequency of cleaning (Moody et al., 2007; Zhai et al., 2008; Hughes and Edwards, 2010). Basically, two main procedures for skin decontamination are described in literature. After exposure to methylene bisphenyl isocyanate, Wester et al. (1999) cleaned the skin of monkeys by subsequent use of five cotton swabs soaked either with water or soap solution, polypropylene glycol, polyglycol-based cleanser or corn oil. Raney and Hope (2006) applied twice a defined volume (500 µl) of water on pig skin, each followed by vigorous refluxing (10 times) and wiping the skin surface with a cotton swab to remove 2 different phospholipids.

An adverse effect of skin cleaning could be an enhancement of dermal penetration ("wash-in" effect) of the chemicals (Moody and Maibach, 2006). For the polycyclic aromatic hydrocarbon benzo(a)pyrene (Moody et al., 1995a) and paraoxon, a metabolite of the pesticide parathion (Misik et al., 2012), a "wash-in" effect was observed. An even up to 32-fold enhanced dermal penetration in different species (pig, rat, human) was observed for the insect repellent N,N-diethyl-m-toluamide (DEET) after cleaning the skin with a water-soap solution followed by water alone (Moody et al., 1995b). Therefore, an adverse "wash-in" effect has to be taken into account in skin decontamination studies warranting the necessity of standardisation of experimental skin cleaning procedures. However, no harmonized experimental protocol is published for *ex vivo* dermal decontamination studies.

In this study, the effectiveness of two skin decontamination techniques was evaluated by the assessment of skin penetration kinetics and the intradermal deposition of 3 hazardous substances with different physicochemical properties.

2. Materials and methods

2.1. Test compounds and their physicochemical properties

1,4-Dioxane, hydrofluoric acid (HF), and anisole were selected as test compounds for percutaneous penetration experiments. 1,4-Dioxane and anisole, both with a purity of ⩾99%, were obtained from Fluka (Buchs, Switzerland) and Sigma–Aldrich (Steinheim, Germany), respectively. 5% aqueous solution of HF (VLSI Selectipur®) was purchased from BASF (Ludwigshafen, Germany).

The physicochemical properties of the test compounds are summarised in Table 1. The data were obtained from the PhysProp® database of Syracuse Research Corporation (SRC, Syracuse, NY, USA; http://www.syrres.com).

2.2. Preparation of skin membranes

Skin from the abdominal area of 4 female donors (age: 26–56 years, mean: 42.8 years) was obtained anonymously from a local clinic immediately after surgical reduction abdominoplasty, according to ethical guidelines of our university. Within a few hours after excision, subcutaneous fat tissue was removed using a scalpel. The skin was immediately wrapped in aluminium foil and stored at −20 °C as described in the experimental protocol of a recent European multi-centre diffusion cell study (van de Sandt et al., 2004).

For percutaneous penetration experiments, the skin was prepared to a thickness of ~0.9 mm using a scalpel and thawed at room temperature. Skin integrity was assessed visually prior mounting the skin on diffusion cells. After equilibration for ~30 min, skin surface temperature was measured by a digital precision thermometer (GMH 1160 with GOF 500 universal probe, type K; Greisinger electronic GmbH, Regenstauf, Germany).

2.3. Percutaneous penetration experiments

Percutaneous penetration of 1,4-dioxane, 5% HF, and anisole was investigated using static PermeGear® diffusion cells (flat flange joint vertical system; 9 mm orifice; exposure area 0.64 cm^2; receptor chamber volume ~5 ml) (SES GmbH, Bechenheim, Germany), which are similar to the cells described by Franz (1975). The receptor chambers were filled with 0.9% aqueous NaCl solution, supplied with a magnetic Teflon stirring bar, and heated at 35 °C during the experiments by a thermostatic circulating water bath (MV-4; Julabo GmbH, Seelbach, Germany).

To control background contamination blank samples of 500 µl receptor fluid were taken from the diffusion cells before the skin was exposed to the test compounds. The sampled volume was immediately replaced by fresh receptor fluid. Percutaneous penetration experiments were performed using 2 skin membranes in parallel from each donor. 100 µl (~156 µl/cm^2) of 1,4-dioxane (c = 161 mg/cm^2), 5% HF (c = 7.5 mg fluoride/cm^2) and anisole (c = 155 mg/cm^2) were applied as single doses without occlusion to the epidermal site of the skin fixed between donor and receptor chamber of diffusion cells.

After one hour, skin surfaces exposed to 1,4-dioxane and anisole were gently wiped with one single dry cotton swab to remove the excess of the compounds before starting the cleaning. Due to its high skin corrosiveness, HF was already removed after 3 min. The cotton swabs were transferred into flange glass vials containing 4 ml of 0.9% aqueous NaCl solution and stored frozen at −20 °C until analysis. The skin cleaning procedures are described in detail in Section 2.4.

Receptor fluid samples (500 µl) were collected during exposure (HF: 3 min; 1,4-dioxane and anisole: 30, 45, and 60 min) and after skin cleaning (0.5, 1, 2, 4, 6, 8 h). The sampled volume was immediately replaced by fresh receptor fluid.

To determine the skin reservoir capacity, the stratum corneum was tape-stripped (10 times) and circular skin punches (ø 10 mm) from the exposure area were taken 8 h after cleaning of the skin. These samples of the skin compartments were digested in closed flange glass vials containing 2 ml of aqueous 1.5 M KOH/ethanol (4:1 v/v) solution and preserved closed at room temperature for a few days. When digestion was completed the samples were analysed.

2.4. Skin cleaning procedures

The effectiveness of two different skin cleaning procedures to remove 1,4-dioxane, HF, and anisole using 500 µl of purified water (Aqua ad iniectabilia; B. Braun Melsungen AG, Melsungen, Germany) was compared. Skin samples, which have not been cleaned after exposure to the test compounds, were used as control.

For the first skin cleaning procedure (skin cleaning I), the skin surface was gently wiped for ~30 s consecutively with 6 moist cotton swabs (one-sided), soaked with a total volume of 500 µl purified water.

For the second skin cleaning procedure (skin cleaning II), the entire volume (500 µl) of the purified water was applied at once into the donor chambers using a pipette. The water was collected