Toxicology in Vitro 27 (2013) 2169–2174

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

The impact of surface loading and dosing scheme on the skin uptake of fragrances

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ARTICLE INFO

Article history:
Received 3 June 2013
Accepted 5 September 2013
Available online 13 September 2013

Keywords:
Fragrance allergy
Fragrance uptake
In vitro skin penetration
Patch test
Repeated open application test
Stratum corneum lipids

ABSTRACT

This study compared the skin uptake of γ-undecalactone, decanol, and dodecyl acetate in an *in vitro*, unoccluded penetration assay in which they were applied to porcine skin at different finite loadings and application schemes. The pattern of fractional uptake differed between the chemicals and did not show the often assumed inverse correlation with surface loading. Furthermore, the mass uptake of identical cumulative amounts of the chemicals was not always additive. These results show that the uptake of fragrances in absence of occlusion and at finite loadings is chemical-specific and depends on the surface loading, the application scheme, and most probably, on the effects of the chemicals on the skin barrier efficiency. The observed lack of additivity might explain some of the differences in the responses observed in patch and repeated open application tests, and the boosting of the allergic state in sensitized individuals by sub-clinical exposures.

1. Introduction

Both the assessment of the sensitization and systemic hazard potentials of chemicals following dermal exposure and the diagnostic tests for contact allergy rely on data on the skin uptake of the chemicals (Griem et al., 2003; Kimber et al., 2001). This *in vitro* study compared the skin uptake of three fragrance materials (FM) applied to the skin at different loadings and dosing schemes. The correlation between fractional skin uptake and surface loading and the influence of the dosing scheme on the mass uptake differed between the chemicals. These results suggest that the structure-specific, dose-dependent interactions between topical chemicals and the skin barrier lipids are an important factor to consider when interpreting the results of the practiced *in vivo* and *in vitro* assays.

Human skin uptake of FM *in vivo* is often estimated from the uptake measured in animal or *in vitro* studies performed at loadings and dosing schemes which differ from those found in real-life exposure conditions (Kimber et al., 2001; Vecchia and Bunge, 2006). The two most commonly practiced diagnostic *in vivo* tests for contact allergy to FM, the patch test (PT) and the repeated open application test (ROAT) (Basketter, 2009; Hannuksela and Salo, 1986), often deliver different numbers of responses to identical cumulative doses of topically applied chemicals and different individual elicitation thresholds (Fischer et al., 2009; Villarama and Maibach, 2004). Several studies have suggested that the reasons behind these apparent discrepancies (Johansen et al., 1996a,b) and the causes for some effects observed in the tests [e.g., the boosting of the allergic state by sub-clinical exposures (Friedmann, 1994; Friedmann et al., 1990)] might be rooted in differences in the dermal uptake of the topically applied chemicals resulting from the different test designs (Fischer et al., 2009; Hostynek and Maibach, 2004). Clearly, detailed knowledge of all parameters that influence the dermal uptake is necessary to correctly evaluate the test results and relevance.

It is widely acknowledged that the surface loading of topically applied chemicals influences their dermal uptake (Buist et al., 2009; Kissel, 2011). The fractional uptake (i.e., the percentage of the applied dose that has been absorbed per unit time) of many chemicals applied to the skin neat or in rapidly evaporating solvents is inversely proportional to their surface loading (expressed as mass per unit area) (Brewster et al., 1989; Wester and Maibach, 1976). This correlation, however, holds only when the chemicals cover the skin surface completely, there is no

Abbreviations: C10, decanol; DA, dodecyl acetate; FM, fragrance material; GC/FID, gas chromatography with flame ionization detector; PBS, phosphate buffer saline; PBST, phosphate buffer saline containing 1% Tween-20; PT, patch test; ROAT, repeated open application test; SC, Stratum corneum; UL, γ-undecalactone.

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http://dx.doi.org/10.1016/j.tiv.2013.09.002

depletion of their source or skin saturation for the duration of the experiment, and they do not modify the skin permeability (Bunge, 2005; Kissel, 2011). These conditions are hardly ever fulfilled during the typical application of fragranced products: the volatile FM are applied to the skin surface at finite loadings (which decrease in the course of the application period because of evaporation of the chemicals) and are not uniformly distributed. Importantly, they might affect the permeability of the skin barrier layer, the Stratum corneum (SC). Several classes of FM (e.g., terpenes, alcohols, fatty acids, and fatty esters) are known to perturb the molecular organization of the SC lipids and/or proteins and thereby enhance the SC permeability to other co-formulated molecules and to themselves (Babita et al., 2006; Babu et al., 2006; Thakur et al., 2006; Williams and Barry, 2004). Since this effect is typically dose-dependent, one high dose of FM might cause a bigger perturbation of the SC permeability and thereby a higher uptake than the cumulative uptake obtained after several applications of smaller doses of FM. Thus, the fractional uptake of FM may depend not only on the surface loading but also on the dosing scheme of the chemical and the correlation between the three parameters is most probably chemical-specific. Indeed, a deviation from the inverse correlation between surface loading and fractional uptake has been reported for a number of known irritants and volatile chemicals (Buist et al., 2009).

In this work, we addressed the correlation between skin uptake, mass loading, and dosing scheme in the skin absorption of three FM—γ-undecalactone (UL), decanol (C10), and dodecyl acetate (DA)—in a 24-h, *in vitro* penetration study using porcine skin. We chose the FM to have different lipophilicities (as measured by their log*P* values) and chemical functionalities, and medium to low volatility. Recently, we have demonstrated that these FM perturb the molecular organization of the SC lipids in a dose-dependent manner (Groen et al., 2013). To mimic the typical in-use conditions for fragrances, we left the skin un-occluded and exposed to the ambient air. We applied the chemicals to the skin surface at six different surface loadings ranging between approximately 20 and 900 μg/cm^2. For the surface loadings of 78, 390, and 780 μg/cm^2 we used two different dosing schemes and applied the chemicals at once or in three consecutive single doses. At the end of the exposure period, we quantified the amounts of the three FM in the viable skin layers (dermis and epidermis) and in the receptor compartment. The sum of these amounts (denoted as systemic uptake) corresponds to the upper limit of the topically applied FM that can reach and interact with the viable skin tissues during the exposure period. To investigate if the correlation between fractional systemic uptake and surface loading was sensitive to the chemical structure of the three FM, we compared their fractional uptake at different surface loadings applied to the skin surface at once. To evaluate the influence of the dosing scheme on the systemic uptake, we next compared the uptake resulting from identical cumulative loadings of the FM applied following different dosing schemes.

2. Materials and methods

2.1. Fragrance materials (FM)

Table 1 shows relevant information on the three FM used in this study. We formulated them as 9:1 (v/v) ethanol/water solutions containing nominally 1%, 5%, and 10% (w/w) of FM. Before use, we quantified the exact concentration of FM in the solutions by GC/FID.

2.2. Skin samples

We collected the ears of domestic pigs at the local slaughterhouse (Gland, Switzerland) a couple of hours *post mortem*. After thoroughly washing the ears with cold water, we removed the skin from the outer sides of the ears using a scalpel, clipped the hairs using hair clippers, dermatomed the skin to a thickness of 350–400 μm using a 50-mm electric dermatome (Nouvag AG, Goldach, Switzerland), and wiped the SC side three times with cotton swabs wetted with cold (4 °C) heptane, to remove traces of sebum. The skin was stored at −20 °C wrapped in Parafilm and packed in Zip-Lock bags for not longer than 1 month prior to use. As recommended in the regulatory guidelines (OECD, 2004; SCCS, 2010), before use we assessed the integrity of the SC using measurement of transepidermal water loss, TEWL (Imhof, 2009). The criterion for barrier integrity that we used throughout this work was of TEWL lower than 12.2 g m^{-2} h^{-1} (as measured by the closed-chamber AquaFlux instrument from Biox Systems, Ltd., UK). We have established that this criterion corresponds to a transcutaneous electrical resistance equal to 14 kΩ cm^2, the most conservative cutoff value reported for porcine ear skin (Davies et al., 2004; data not shown).

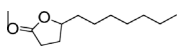
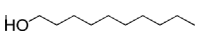
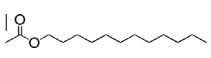
2.3. Diffusion cells

We used static jacketed, Franz-type, glass diffusion cells (PermeGear, Inc., Hellertown, USA) with a receptor volume of 8 mL, equipped with magnetic stirrers. Before use, the cells were cleaned thoroughly, plasma-activated, and gas-phase silanized with trichloro perfluorooctyl silane. As a receptor solution we used PBS buffer (150 mM, pH 7.4) containing 1% Tween-20 (denoted as PBST). All studied FM were soluble in this solution at concentrations exceeding their maximal possible concentrations (i.e., those corresponding to the total amount of applied FM dissolved in 8 mL, the volume of the receptor compartment).

2.4. Experimental design

We performed all experiments in an air-conditioned room with a temperature of 25 ± 2 °C, relative humidity of 50 ± 4%, and average air velocity above the bench top of ⩽0.1 m/s. At beginning of the experiments, we defrosted the skin samples for 30 min on

Table 1
Fragrance materials used in this study.

Fragrance material	CAS no.	Structure	Molecular weight	log*P*	Vapor pressure
γ-Undecalactone (UL)	000104-67-6		184.28	3.06	0.55
Decanol (C10)	000112-30-1		158.28	4.57	1.45
Dodecyl acetate (DA)	000112-66-3		228.37	5.78	1.61