Regulatory Toxicology and Pharmacology 91 (2017) 197–207

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Application of *in vitro* skin penetration measurements to confirm and refine the quantitative skin sensitization risk assessment of methylisothiazolinone

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ARTICLE INFO

Keywords:
Methylisothiazolinone
Quantitative-risk-assessment
Measured exposure levels
Skin penetration
Cosmetics
Household care
Consumer
Skin sensitization

ABSTRACT

Use of quantitative risk assessment (QRA) for assessing the skin sensitization potential of chemicals present in consumer products requires an understanding of hazard and product exposure. In the absence of data, consumer exposure is based on relevant habits and practices and assumes 100% skin uptake of the applied dose. To confirm and refine the exposure, a novel design for *in vitro* skin exposure measurements was conducted with the preservative, methylisothiazolinone (MI), in beauty care (BC) and household care (HHC) products using realistic consumer exposure conditions. A difference between measured exposure levels (MELs) for MI in leave-on versus rinse-off BC products, and lower MELs for MI in HHC rinse-off compared to BC products was demonstrated. For repeated product applications, the measured exposure was lower than estimations based on summation of applied amounts. Compared to rinse-off products, leave-on applications resulted in higher MELs, correlating with the higher incidences of allergic contact dermatitis associated with those product types. Lower MELs for MI in rinse-off products indicate a lower likelihood to induce skin sensitization, also after multiple daily applications. These *in vitro* skin exposure measurements indicate conservatism of default exposure estimates applied in skin sensitization QRA and might be helpful in future risk assessments.

1. Introduction

A good understanding of hazard as well as consumer exposure to a chemical present in a beauty care (BC) or household care product (HHC) is required to evaluate the potential risk of inducing skin sensitization in consumers. Some ingredients in consumer products that are known to have skin sensitization potential can be safely formulated as long as skin exposure to them is sufficiently lower than their defined threshold for inducing contact allergy (Felter et al., 2003; Api et al., 2008). Determining accurate consumer exposure levels can be very challenging as standard exposure values are based mainly on the total dose applied externally without consideration of the exact amount relevant for skin sensitization. For aggregate exposures, simple summation of values based on repetitively externally applied doses are anticipated to be unrealistic as this tends to overestimate exposure. Standard *in vitro* absorption studies routinely examine a single chemical or product exposure at a time, determining usually the systemic exposure (e.g. the amount present in the receptor fluid). This standard study design does not meet the requirements for measuring the skin exposure under consumer relevant usage conditions for HHC or BC products. In reality, consumers commonly experience complex repeated exposure scenarios to multiple product types/matrices with cycles of product removal. When measuring consumer exposure under product use conditions, a single application of the products is done for most situations, while some exposure scenarios require the repetitive application of products, such as multiple usage of e.g. a hand soap during a single day, or beauty salon professionals' exposure to ingredients (e.g. preservatives) in shampoos, conditioners, and body care products used during their work day. Standard *in vitro* dermal penetration studies using human or pig skin are designed to reflect operational exposure scenarios (OECD, 2004a and b; SCCS, 2010) and typically measure the exposure to a single applied amount at a fixed dose of a chemical over 24 h, which is entirely unrelated to the amount used in a product formulation or the actual exposure time during product use. However, the study design can be adapted to best simulate different exposure scenarios, including simulation of rinse-off or leave-on products, as well as

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http://dx.doi.org/10.1016/j.yrtph.2017.10.024
Received 18 July 2017; Received in revised form 17 October 2017; Accepted 22 October 2017

repeated application of products over periods up to 96 h. Exposures determined in those modified skin exposure studies can also inform the quantitative risk assessment for skin sensitization (QRA) and can be used as replacement for consumer exposure calculations based on Habits & Practice (H & P) data.

The QRA approach for skin sensitization is based on the determination of the amount of chemical per unit area of exposed skin that is not expected to result in the induction of skin sensitization (e.g. the 'No Expected Sensitization Induction Level' or NESIL) followed by the application of sensitization-assessment-factors (SAFs) (Gerberick et al., 2001; Api et al., 2008) to derive the acceptable exposure level (AEL) for consumers. The NESIL is derived using a weight of evidence approach, and is often based on data from a local lymph node assay (LLNA) or a well-conducted confirmatory Human Repeat Insult Patch Test (HRIPT). The SAFs are used to extrapolate from the experimental conditions of the NESIL (defined and controlled exposure) to real life consumer exposure (variable exposure controlled by the consumer) and to account for variations between subjects, matrices (other components causing skin irritation or enhanced skin penetration), and product use patterns affecting exposure considerations e.g. frequency, occlusion and skin condition or body sites (Felter et al., 2002; Api et al., 2008; Basketter and Safford, 2016). The QRA approach for contact allergens in consumer products compares the calculated consumer exposure level (CEL, expressed as dose per unit area (μg/cm^2)) to the ingredient of interest in the product, with an AEL. When the AEL/CEL ratio is greater than 1, the exposure to the ingredient is unlikely to result in the induction of skin sensitization. More recently, the use of actual measured exposures to compounds applied to the skin under relevant exposure conditions, namely the "Measured (consumer) Exposure Level" (MEL) (Goebel et al., 2010) has been used in the QRA, replacing or refining the CEL. The MEL is the total amount of chemical recovered in the epidermis, dermis and receptor fluid (RF) plus amounts found in the stratum corneum (SC) when applied under consumer relevant conditions, at the end of the exposure (i.e. after rinsing, or drying). While this gives a realistic picture of the actual skin exposure, it is still a conservative approach since, normally, the SC is not considered to contribute to biological effects involved in skin sensitization (SCCS, 2010). This concept uses *in vitro* skin exposure measurements as a refinement for the applied dose in the context of skin sensitization QRA, and has been used to evaluate the skin sensitizing potential of certain hair dye ingredients (Goebel et al., 2012, 2014). This QRA approach involves a direct comparison of the NESIL (or the AEL) with the MEL. When applying a MEL instead of a CEL to inform the QRA, one needs to ensure that the NESIL and, subsequently the AEL, may also require adequate adjustment from the applied dose. Correct exposure information is critical and provides reassurance of the QRA and with that supports the risk assessment decisions.

Preservatives are key ingredients in consumer, professional and industrial products, serving to not only prevent microbial contamination of the product itself but also to protect the consumer from adverse effects including infection resulting from use of contaminated products. However, because of their anti-microbial activity, preservatives are naturally reactive substances and are often responsible for inducing contact allergy (Yim et al., 2014; Beene et al., 2017). Balancing their use concentration with their efficacy is important since too high concentrations may lead to the induction of skin sensitization (Basketter et al., 2008); while concentrations that are too low will render it ineffective as a preservative. Methylisothiazolinone (MI), a preservative, is a strong human and animal skin allergen (Roberts, 2013; Castanedo-Tardana and Zug, 2013) which is also classified as a skin sensitizer (1A, H317 according to GHS/CLP, CLP regulation, 2008) and has been identified as the causative agent in numerous cases of allergic contact dermatitis (Lundov et al., 2013; Castanedo-Tardana and Zug, 2013). Given the widespread use of MI in a large number of consumer products it is difficult to identify the products which have contributed the most to the outbreak of the MI skin allergy epidemic in Europe and beyond. Until recently the use of MI in cosmetics in Europe was restricted to an upper use limit of 100 ppm (0.01%) (SCCNFP, 2004) for rinse-off and leave-on products *via* the Annex V of the cosmetic directive. It was subsequently banned from leave-on products and its use concentration in rinse-off cosmetic products was reduced up to a maximum of 15 ppm (SCCS, 2013). The concentration of MI in non-cosmetic products such as paints or household care products (all rinse-off products, or not intended to be in contact with skin) has been reported to range from 0.7 to 180.9 ppm, with most products containing MI at 100 ppm or lower (Schwensen et al., 2015; Garcia-Hildago et al., 2017). Classification and labelling restrictions for non-cosmetic products are currently finalised to ensure safe usage of MI in those products.

When a QRA was conducted for a number of cosmetic products with 100 ppm of MI, it was concluded that for the majority of leave-on products, including wet baby wipes, there was a risk for inducing skin sensitization in consumers (i.e., AEL/CEL < 1), and MI should clearly not be used for these product categories at 100 ppm (CIR, 2014) or lower levels which result in unfavourable AEL/CEL ratios. For rinse-off products such as shampoos, hair conditioners and bath soaps, the QRA provides a favourable AEL/CEL ratio > 1, which supports the use of 100 ppm MI in those product types. In addition, applying the QRA methodology to HHC products with MI concentrations of 100 ppm or lower, produces an AEL/CEL ratio > 1, which indicates a low risk for the induction of sensitization due to the use of those products (e.g., laundry products (Kwon et al., 2009)). Exposure to HHC products is generally lower than to cosmetic rinse-off products as skin exposure is not intended and rinsing of exposed skin areas is done thoroughly (Corea et al., 2006). While there is usually an adequate margin of safety for MI in HHC rinse-off products, there are limited data available to confirm the exposure assumptions and the overall risk assessments. Hence, for this investigation MI was chosen to demonstrate how measurement of the skin exposure (determined as MEL) can be used to confirm and possibly refine the QRA by providing a more accurate determination of the in-use skin exposures. When comparing the actual measured value (MEL) with the calculated CEL, if the MEL values are in line or lower than CELs, then the safety assessment for MI present in different cosmetic and household care products gains additional support.

2. Materials and methods

2.1. Materials

[^{14}C]-Methyl isothiazolinone ([4,5-^{14}C]-RH-573); [^{14}C]-MI; with specific activity of 5.57 mCi/mmol (legs 2–4)), was purchased from Rohm and Haas Research Laboratories, Spring House, PA, USA. [^{14}C]-MI with specific activity of 58 mCi/mmol (legs 1, 5–10) was purchased from Quotient Bioresearch, Cardiff, UK Water. Various product formulations were spiked with [^{14}C]-MI at a final concentration of 100 ppm (0.1 g/l). The product formulations used were either commercially available Procter & Gamble product variants without MI (face cream, liquid hand soap, liquid laundry detergent) or products made specifically for this study based on the formulas of commercial Procter & Gamble products (shampoo and conditioner, hand dish washing liquid). All products are representative formulations for their respective product category using standard cosmetic or household care ingredients. (It is recognized that, for the product types examined in this study, formulation differences may impact the skin penetration of MI.)

The [^{14}C]-MI spiked formulations were then used neat or further diluted depending on the habits & practices (H & P) data (Supplementary Table 1 Coty H & P study, HERA, 2005; SCCS, 2015) for each of the use scenarios (Table 1). All preparations for scenarios 1–6 (shampoo, conditioner, hand soap and face cream), as well as for scenarios 9 and 10 (water dilution for HRIPT), were made with 0.1 g/l MI and were used neat; For scenario 7, liquid laundry detergent rinse-off,