Regulatory Toxicology and Pharmacology 87 (2017) 106–111

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Margin of safety of pentylene glycol derived using measurements of cutaneous absorption and volatility

A. Steiner, K. Kugarajan, M. Wullimann, B. Ruty, G. Kunze*

Nestlé Skin Health R&D, Froschackerstrasse 6, CH-4622 Egerkingen, Switzerland

ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form
12 April 2017
Accepted 4 May 2017
Available online 5 May 2017

Keywords:
Skin penetration
Pentylene glycol
Sunscreen
Volatile
Margin of safety
Safety assessment

ABSTRACT

The safety assessment of pentylene glycol (PG) has been based on a bioavailability extrapolated from those of other 1,2-glycols or an assumed 100% absorption. To make a better safety assessment and an accurate calculation of the margin of safety (MoS), the skin penetration of PG present in a commercially available sunscreen was measured in pig skin at different exposure durations. The mass balance of PG decreased with increasing exposure durations, from 98% (1 h) to 29% (24 h) and the amount of PG detected in the skin wash decreased over time from 93% to 3%. The decrease in mass balance was attributed to an unexpected volatility of PG, which was confirmed in additional experiments. The maximum bioavailable amount of PG was 123 μg/cm2 after 24 h and was considered to be worst case scenario (10 mg/cm^2 i.e. 5-fold the recommended application standard dose, 2 mg/cm^2). MoS values for the application of a standard dose of sunscreen after 1–24 h exposure were 140–671 in adults and, if calculated for children ratios, 87–217 Based on the available toxicological data for PG in comparison to the amounts determined to be potentially bioavailable, PG in the test sun protection product SPF 50 + does not show any safety concerns for daily usage at the recommended dosage of 2 mg/cm^2 or lower.

1. Introduction

Pentylene glycol (PG, synonym 1,2-pentanediol, $C_5H_{12}O_2$) is a synthetic member of the 1,2-glycols used in cosmetics and pharmaceutical products. In addition to its skin hydrating effect, it is also known to support antimicrobial protection and serves as a solvent and moisturizing agent (CIR, 2011; Hiroya, 2006; Lee et al., 2011). PG is a clear liquid and can be used within a pH range of 2–12, with a good solubility in water and oil, making it ideal for use in a broad range of products. A survey in 2010 indicated that PG was being used in personal care products at concentrations between 0.001 and 5% (Personal Care Products Council, 2010), which can be regarded as the maximum recommended concentration.

The focus of these studies was on the use of PG as an ingredient of a commercially available sunscreen (type oil-in-water (O/W) with a SPF 50+) as part of the mandatory ingredient/product safety assessment based on the Scientific Committee on Consumer Safety (SCCS) recommended procedure (SCCS, 2015). These evaluations are an essential part of the product information file (PIF) for cosmetic products (EU cosmetic directive 1223/EC/2009). The safety assessments of short and long-chain 1,2-glycols have been based on properties of propylene glycol since it was considered unlikely that longer chain lengths of the carbon backbone would increase the potential for toxicity (CIR, 2011). PG, along with other 1,2-glycols, was considered to be safe as cosmetic ingredients under normal use practices and concentrations. Despite this, there is a lack of comprehensive skin absorption of ingredients present in sunscreens following topical application (Kurul and Hekimoğlu, 2001). Information on the extent of skin penetration of PG has a large impact on the calculation of the margin of safety (MoS) of this chemical. Until now, the bioavailability of PG was based on skin penetration values of chemicals with similar structures (such as 1,2-butanediol or propylene glycol), or calculations of maximal flux using maximum aqueous solubility and LogP values (Kroes et al., 2007). Alternatively, if it is assumed that 100% of PG is absorbed (on the basis that no reliable data is available), the resulting MoS for a solar product would not fall within the SCCS criteria of a safe product (i.e. ≥3.3% ingredient concentration and excluding evaporation information). Information on the skin penetration of PG after topical application is limited, although skin penetration of 1,2-

* Corresponding author. Spirig Pharma AG, Preclinical R&D, Froschackerstrasse 6, CH-4622 Egerkingen, Switzerland.
E-mail address: gernot.kunze@galderma.com (G. Kunze).

http://dx.doi.org/10.1016/j.yrtph.2017.05.007
0273-2300/

glycols has been shown to decrease with increasing chain length (Lee et al., 2011). Studies measuring the penetration of caprylyl glycol (structurally similar to PG) reported a low mass balance (only 50–55% of the applied dose was recovered at the end of the experiment) and attributed the loss to metabolism in the skin and/or chemical degradation (Johnson et al., 2012). While the physicochemical properties of PG (Table 1) suggest it is unlikely to bind with skin proteins (causing a loss in mass balance), the vapor pressure does not indicate that PG could evaporate from the surface of the skin. Thus, there is a gap in the data needed for the safety assessment of this chemical. To address this, we have measured the penetration of PG through pig skin after the application of an example sun protection product containing this ingredient. PG is used in this O/W formulation with SPF 50 + at a concentration of 4%. A typical sun protection product is applied at 9 g per application, twice a day (based on Cosmetic facts sheet RIVM report (RIVM, 2006), and implemented in the SCCS's Notes of Guidance (SCCS, 2015)), resulting in a calculated maximum usage of 18 g per day; therefore, we have selected this product type as a "worst-case safety assessment scenario", rather than a body lotion, which is assessed with a total of 7.82 g per day.

In vitro skin penetration methods have been used for many years for measuring the absorption of topically applied compounds across full- or split-thickness animal (Marti-Mestres et al., 2007) or human (Dayan, 2009) skin to a receptor fluid reservoir below. These methods allow for the estimation of systemic exposure to humans in an overestimated and conservative way when compared with the NOAEL of repeated-dose toxicity studies (Nohynek et al., 2010), and thus, the calculation of a MoS. Among several animal species, pig skin from flank and back represents a reliable model for human skin (Bartek et al., 1972). The experimental conditions were designed to reflect the use conditions for humans when applying a typical sun screen formulation, with the longest exposure duration of 24 h as a worst case scenario. The amount of sunscreen formulation applied for *in vivo* determination of the sun protection factor (SPF) is 2 mg/cm^2 (Colipa et al., 2006). In our experimental setup, a 5-fold higher amount was used (10 mg/cm^2). We have used frozen skin in our experiments since permeability properties of skin are usually maintained after excision from the body and appropriate storage in a freezer for several months (Zalko et al., 2011; Dennerlein et al., 2013). Furthermore, penetration is driven by passive diffusion and there is no evidence for active transport (OECD, 2011).

A main focus of our current studies was to address the loss of mass balance reported by others for similar chemicals and to build a safety assessment using the penetration data.

2. Materials and methods

2.1. Materials

The test item was a commercially available sun protection product with SPF 50+, containing 4% PG (CAS No. 5343-92-0), and was from Galderma, manufactured by Spirig Pharma AG.

Pig (Schweizer Landedelschwein, obtained from a local butchery) back and flank skin from three donors (12 skin discs per donor, of which 9 were used for the measurement of skin absorption of PG), were dermatomed to a thickness of approximately 1 mm (1.15 ± 0.13 mm) and stored at −20 °C until use.

Table 1
Registration identifier and physicochemical properties of PG. The values were from ACD Labs software (ACD, 2010).

Property/Identifier	Value
EC number	226-285-3
CAS number	5343-92-0
Molecular weight	104.2
Solubility	95 g/l (pH 7, 25 °C)
Vapor pressure	5.75 × 10-02 Torr (25 °C)
pKa	14.2 ± 0.20 (25 °C)
logP	−0.278 ± 0.215 (25 °C)

2.2. Experimental design

2.2.1. Skin absorption method

The skin absorption of PG following the application of test product was performed using a Franz cell static system, according to the OECD Guideline No. 428 (OECD, 2004) and Diembeck et al. (1999). The receptor fluid, pH = 7.3, was 0.14 M NaCl, 2 mM K_2HPO_4 and 0.4 mM KH_2PO_4.

Thawed skin samples were mounted in diffusion chambers maintained at 34 ± 0.3 °C. There were 3 independent experiments carried out using 3 discs for each time point such that a total of 9 skin disks were tested per time point. The application area was 1.77 cm^2. The skin integrity was analyzed after an equilibration period of 1 h according to the transepidermal water loss (TEWL), using a Tewameter (Tewameter TM 300, CK electronic GmbH, 50829 Köln/Germany). TEWL values (9 discs per time point) were within the typical range for porcine skin (2–15 g/h/m^2 (Davies et al., 2015)).

Although the recommendation for a solar protection product (based on EU Cosmetic directive 2006/647/EG L 265/39) is 2 mg/cm^2; the application of this dose of a semi-solid and highly viscous formulation to a small test areas (i.e. the skin in a Franz cell and/or Eppendorf lids) is technically challenging, as also acknowledged in the SCCS Notes of guidance for the testing of cosmetic ingredients and their safety evaluation (9th Edition, SCCS 1564/15). Therefore, in order to achieve reproducibility in the application of the sunscreen product, we increased the dose to 10 mg/cm^2 and applied it using a spatula.

The product was exposed to the skin for 1, 3, 6 and 24 h. After each time point, the experiment was terminated and the cutaneous distribution of PG measured. At the end of the exposure period, the formulation was washed from the skin surface in five steps (5 × 1 ml of receptor fluid).

Receptor fluid samples were filtered with a Titan2 HPLC Reg. Cell. 0.2 μm/30 mm filter and analyzed by gas chromatography. The Franz cell lid, the skin wash and the tips used for washing and drying the skin (rinsed with 1 ml of acetonitrile 50%) were collected in a 50 ml glass beaker. A volume of 24 ml of receptor fluid was then added. Extraction of PG was achieved by heating samples to 60 °C in an ultrasonic bath for 30 min. After the wash had cooled to room temperature, it was filtered with a Titan2 PTFE HPLC filter before analysis by gas chromatography. After the removal of the receptor fluid and the wash step, the whole skin was dissolved in 10 ml 50% acetonitrile. The samples were vortexed vigorously and incubated for 1 h at 60 °C (with a shake every 15 min) and then filtered with a Titan2 PTFE HPLC filter. All samples were stored at 4 °C, if not processed immediately. Samples used for the determination of the stability in receptor fluid were stored at room temperature until analysis.

2.2.2. Determination of evaporation of PG

An overview of the method used to determine the evaporation potential of PG is shown in Fig. 1. The test product was applied on the inner surface of a 5 ml polypropylene Eppendorf tube lid. The application amount of 14.1 mg corresponds to 10 mg/cm^2, as used in the skin absorption study. The lids were either left open (pre-cut from the Eppendorf tubes, n = 3) or closed (n = 3) and then