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IN CHEMICO SKIN SENSITIZATION STUDY – STEARAMIDOPROPYL DIMETHYLAMINE

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1. INTRODUCTION

Cardno ChemRisk was asked by WEN By Chaz Dean, Inc. ("WCD"), to conduct a comprehensive risk and safety assessment of the cosmetic product commonly known as WEN® by Chaz Dean Cleansing Conditioner (the "WEN Products"), and, specifically, whether the product causes hair loss and/or any other adverse dermal event, which evaluation was triggered by complaints and allegations that the WEN Products caused hair loss in a very small percentage of consumers. As part of that comprehensive risk and safety assessment, we reviewed the ingredients and constituents in the WEN Products to identify ingredients that had the potential to cause adverse dermal reactions in skin. One such ingredient was stearamidopropyl dimethylamine.

Cardno ChemRisk utilized the Organisation for Economic Co-operation and Development (OECD) 442C *in chemico* sensitization testing guideline: Direct Peptide Reactivity Assay (DPRA) to evaluate the skin sensitization potential of stearamidopropyl dimethylamine. The OECD is an international respected intergovernmental economic organization that provides its members with a forum and a platform to compare policy experiences, seek answers to common problems, identify good practices and coordinate domestic international policies of its members which publishes guidelines for various industries on good practices. One such guideline that it has published is the 442C that evaluates the protein reactivity of a test article by quantifying the reactivity of test chemicals toward model synthetic peptides containing either lysine or cysteine (OECD 442C; Gerberick et al. 2004). The percentage of cysteine and lysine peptide depletion are then used to categorize a substance in one of four classes of reactivity for supporting the discrimination between skin sensitizers and non-sensitizers (OECD 442C; Gerberick et al. 2007). The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) considered this test to be scientifically valid and noted that it can be used to "support the discrimination between skin sensiti[z]ers and non-sensiti[z]ers for the purpose of hazard classification and labelling" (OECD 442C). It is important to note that the results from this test alone may not be sufficient to conclude the skin sensitization potential of a test article as protein reactivity only represents one step in the multistep process of skin sensitization (OECD 442C).

2. BACKGROUND

Stearamidopropyl dimethylamine is physically characterized as a waxy flake (Belsito et al. 2014). Formulations of stearamidopropyl dimethylamine may contain dimethylaminopropylamine (DMAPA) as an impurity from the manufacturing process (Belsito et al. 2014). DMAPA was reported to have sensitizing potential (Belsito et al. 2014). Stearamidopropyl dimethylamine functions as an antistatic agent and hair conditioning agent in cosmetics (Belsito et al. 2014). According to the FDA's Voluntary Cosmetic Registration Program (VCRP), stearamidopropyl dimethylamine was registered for use in over 400 cosmetic and personal care products; the majority of the listed products were rinse-off formulations such as hair conditioners (Belsito et al. 2014). The maximum concentrations of stearamidopropyl dimethylamine reported in personal care products ranged from 0.01% to 5% (Belsito et al. 2014). The CIR concluded that stearamidopropyl dimethylamine was considered "safe in cosmetics when they are formulated to be non-sensitizing" (Belsito et al. 2014).

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2.1 Skin Sensitization

A skin sensitizer is "a substance that will lead to an allergic response following skin contact" (OECD 442C). Generally, skin sensitization induction is a multistep process starting with a covalent binding of a constituent with skin proteins, which leads to a series of immune responses resulting in allergic contact dermatitis and contact hypersensitivity (OECD 442C).

Allergic contact dermatitis (ACD) is a common inflammatory skin disease that typically develops due to prolonged or repeated exposure to chemical allergens (Gober et al. 2008; Becker 2013; Thyssen et al. 2014). An estimated 15 to 20% of the general population suffers from ACD to at least one chemical; common allergens include metals, fragrances, and preservatives (Nelson et al. 2010; Martin 2012). Identified risk factors include sex (a higher frequency of ACD is observed in women), age (frequent onset at young age), occupational exposure, exposure from consumer products, and genetic predisposition (Martin 2012). Patients with ACD usually present with well-defined eczematous dermatitis characterized by redness, swelling, itching, and blistering of the affected skin (Saint-Mezard et al. 2004; Nelson et al. 2010; Basketter et al. 2015).

ACD is driven by a form of delayed-type hypersensitivity reaction resulting from prior sensitization to the inducing contact allergen (Basketter et al. 2015). The immune-mediated process is made up of two distinct phases: an induction (or sensitization) phase and an elicitation phase (Saint-Mezard et al. 2004; Gober et al. 2008). Small molecular compounds (haptens) that cause ACD chemically react to endogenous protein within the skin during the induction phase, rendering the molecule antigenic (Gober et al. 2008; Martin 2012). During the elicitation phase, haptens diffuse in the skin and are recognized by the patient's immune system resulting in an inflammatory response, leading to the aforementioned dermatitis symptoms (Saint-Mezard et al. 2004; Gober et al. 2008).

Damage to the hair can occur when personal care or cosmetic products are used incorrectly or too frequently, which may produce changes in hair texture that correspond to morphologic changes or even hair loss (Ahn and Lee 2002). Identified examples of such occurrences typically involve skin irritation and sensitization. For example, irritation to the skin may occur when irritants and allergens from cosmetics, such as hair dye, penetrate the scalp (Ishida, Makino et al. 2011; AlGhamdi and Moussa 2012). Alghamdi and Moussa, (2012) reported that hair loss was a side effect among individuals who experienced skin irritation as a result of the use of hair dyes. In addition, hair highlighting has been shown to be able to cause allergic and irritant contact dermatitis resulting in hair loss (Lund, Unwala et al. 2010). Researchers have also reported cases of inflammatory alopecia and allergic contact dermatitis following topical triggers, such as fragrances, sunscreens, as well as personal care and cosmetic products (Aldoori, Dobson et al. 2016; Admani, Goldenberg et al. 2017; Liu, Zimarowski et al. 2017). Goldenburg et al., (2017) noted that the "hallmark for contact alopecia is a preceding eczematous localized inflammatory response followed by hair loss, with notable regrowth of hair occurring by 6 months after allergen avoidance…[which is] consistent with contact-associated telogen effluvium" (Goldenberg, Admani et al. 2017: p. 626). Accordingly, based on the literature, hair loss caused by a cosmetic product would not be expected to occur without symptoms of irritation or sensitization.

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3. METHODOLOGY

A solution of 1% stearamidopropyl dimethylamine was prepared in acetonitrile and corrected to 100 mM, per OECD 442C guideline test requirement. This represents an effective concentration of 2.1% stearamidopropyl dimethylamine in water. Thus, all conclusions from this study pertain to an effective concentration of 2.1% stearamidopropyl dimethylamine.

Cysteine or lysine-containing peptide solution was incubated in triplicate with stearamidopropyl dimethylamine, a negative control (phosphate buffered saline), or a positive control (100mM cinnamic aldehyde solution) for 24 hours at 25°C. After 24 hour incubation, relative peptide concentration was quantified by high performance liquid chromatography (HPLC) with gradient elution and ultraviolet (UV) detection at 220 nm. Several calibration curves were generated from analyses of standard solutions of cysteine and lysine peptides. Cysteine and lysine peptide percent depletion values were calculated.

The overall mean cysteine and lysine percent depletion values were then classified following the cysteine 1:10/lysine 1:50 prediction model from the OECD RTG 442C guideline (Table 1):

Table 1. Cysteine 1:10/Lysine 1:50 Prediction Model

Mean Cysteine and Lysine % Depletion	Reactivity Class	DPRA Prediction
0% ≤ mean % depletion ≤ 6.38%	No or minimal reactivity	Negative
6.38% ≤ mean % depletion ≤ 22.62%	Low reactivity	Positive
22.62%≤ mean % depletion ≤ 42.47%	Moderate reactivity	
42.47% ≤ mean % depletion ≤ 100%	High reactivity	

According to Table 1, a threshold value of 6.38% average cysteine and lysine peptide depletion can be used to differentiate between skin sensitizers and non-sensitizers.

This test is considered to be valid if 1) the calibration curve has an r^2 value > 0.99, 2) the coefficient of variation (CV) of peptide peak areas for reference controls is <15%, and 3) if the mean percent depletion values of the three positive control replicates are between 60.8 to 100% with a standard deviation (SD) of <14.9% for the cysteine peptide and between 40.2 to 69.0% with an SD of <11.6% for the lysine peptide.

4. RESULTS AND DISCUSSION

Stearamidopropyl dimethylamine was evaluated for peptide reactivity, an initiation event in the multistep induction phase of skin sensitization. Results for stearamidopropyl dimethylamine and positive controls are reported in Tables 2 and 3. The reactivity class and DPRA prediction according to the OECD 442C guideline is reported in Table 4.

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Table 2. Cysteine Assay Results for Stearamidopropyl Dimethylamine and Positive Control

Sample ID	% Cysteine Depletion	Mean % Cysteine Depletion	Std. Dev.	CV
Stearamidopropyl dimethylamine – 1	-0.2	1.2	1.3	108%
Stearamidopropyl dimethylamine – 2	1.1			
Stearamidopropyl dimethylamine – 3	2.7			
Positive Control – 1	66.1	66.4	0.3	0.4%
Positive Control – 2	66.3			
Positive Control – 3	66.3			

Table 3. Lysine Assay Results for Stearamidopropyl Dimethylamine and Positive Control

Sample ID	% Lysine Depletion	Mean % Lysine Depletion	Std. Dev.	CV
Stearamidopropyl dimethylamine – 1	0.5	0.4	0.1	29%
Stearamidopropyl dimethylamine – 2	0.4			
Stearamidopropyl dimethylamine – 3	0.3			
Positive Control – 1	56.5	54.0	2.1	3.9%
Positive Control – 2	52.9			
Positive Control – 3	52.8			

Table 4. Reactivity Class and DPRA Prediction for Stearamidopropyl Dimethylamine

Test Substance	Concentration	Reactivity Class	DPRA Prediction
Stearamidopropyl dimethylamine	2.1%	No or minimal reactivity	Negative

Briefly, the mean % cysteine peptide depletion for the positive control was 66.4% with a SD of 0.4% (Table 2). The mean % lysine peptide depletion for the positive control was 54% with a SD of 3.9% (Table 3). According to the OECD 442C test guideline, these positive control values fell within the specified parameters and thus this test is considered valid. The mean % cysteine peptide depletion for stearamidopropyl dimethylamine was 1.2% with a SD of 108% (Table 2). The mean % lysine peptide depletion for stearamidopropyl dimethylamine was 0.4% with a SD of 29% (Table 3). According to the OECD 442C test guideline, stearamidopropyl dimethylamine has no or minimal reactivity and is predicted to be negative for skin sensitization (Table 4).

5. CONCLUSION

Cardno ChemRisk conducted an *in chemico* skin sensitization study on stearamidopropyl dimethylamine. Stearamidopropyl dimethylamine displayed no or minimal peptide reactivity at the evaluated concentration of 2.1%. According to the OECD 442C test, stearamidopropyl dimethylamine would not be expected to be skin sensitizing at 2.1%. Specifically, the molecular

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initiating event of skin sensitization would not be induced at a stearamidopropyl dimethylamine concentration of 2.1%. [redacted] Therefore, the concentration of stearamidopropyl dimethylamine in the WEN Products would not be expected to induce skin sensitization in a consumer.

6. REFERENCES

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