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IN CHEMICO SKIN SENSITIZATION STUDY – PANTHENOL

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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 panthenol.

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 panthenol. 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

Panthenol is a pro-vitamin alcohol analogue to pantothenic acid that is rapidly converted to pantothenic acid in the body (Vitamin B5) (Camargo Jr et al. 2011; Stables et al. 1998). It is a viscous, hygroscopic liquid that is soluble in both water and alcohol (Johnson 1987). It is widely used in the pharmaceutical and cosmetic industry due to its moisturizing, soothing, sedative, and healing properties (Camargo Jr et al. 2011; Chin et al. 2013). Topical application of panthenol was reported to aid healing of burns, fissures, lesions, and allergic dermatitis (Camargo Jr et al. 2011). According to an early review, the concentration range of panthenol used in cosmetics was between 0.1 to 1% with a small number of formulations that used up to 25% (Johnson 1987). The authors concluded that panthenol was considered to be "safe as presently used in cosmetics" (Johnson 1987). Furthermore, panthenol was generally recognized as safe (GRAS) when used as a dietary supplement (Johnson 1987).

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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% panthenol was prepared in acetonitrile and corrected to 100 mM, per OECD 442C guideline test requirement. This represents an effective concentration of 2.2% panthenol in water. Thus, all conclusions from this study pertain to an effective concentration of 2.2% panthenol. For comparison, panthenol is present at a maximum concentration of 1% in WCD cleansing conditioners.

Cysteine or lysine-containing peptide solution was incubated in triplicate with panthenol, 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

Panthenol was evaluated for peptide reactivity, an initiation event in the multistep induction phase of skin sensitization. Results for panthenol 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 Panthenol and Positive Control

Sample ID	% Cysteine Depletion	Mean % Cysteine Depletion	Std. Dev.	CV
Panthenol – 1	-2.3	0.6	0.8	130%
Panthenol – 2	0.3			
Panthenol – 3	1.6			
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 Panthenol and Positive Control

Sample ID	% Lysine Depletion	Mean % Lysine Depletion	Std. Dev.	CV
Panthenol – 1	0.4	0.2	0.2	71.6%
Panthenol – 2	0.1			
Panthenol – 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 Panthenol

Test Substance	Concentration	Reactivity Class	DPRA Prediction
Panthenol	2.2%	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 panthenol was 0.6% with a SD of 130% (Table 2). The mean % lysine peptide depletion for panthenol was 0.2% with a SD of 71.6% (Table 3). According to the OECD 442C test guideline, panthenol 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 panthenol. Panthenol displayed no or minimal peptide reactivity at the evaluated concentration of 2.2%. According to the OECD 442C test, panthenol would not be expected to be skin sensitizing at 2.2%. Specifically, the molecular initiating event of skin sensitization would not be induced at a panthenol concentration of 2.2%. [redacted] Therefore, the concentration of panthenol in the WEN Products would not be expected to induce skin sensitization in a consumer.

6. REFERENCES

Basketter, D., I. White, J. McFadden, and I. Kimber. 2015. Skin sensitization: implications for integration of clinical data into hazard identification and risk assessment. *Human & experimental toxicology* 34 (12):1222-1230.

Becker, D. 2013. Allergic contact dermatitis. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* 11 (7):607-621.

Camargo Jr, F. B., L. R. Gaspar, and P. M. Maia Campos. 2011. Skin moisturizing effects of panthenol-based formulations. *Journal of cosmetic science* 62 (4):361.

Chin, M. F., T. M. Hughes, and N. M. Stone. 2013. Allergic contact dermatitis caused by panthenol in a child. *Contact Dermatitis* 69 (5):321-322.

Gerberick, G. F., J. D. Vassallo, R. E. Bailey, J. G. Chaney, S. W. Morrall, and J.-P. Lepoittevin. 2004. Development of a peptide reactivity assay for screening contact allergens. *Toxicological Sciences* 81 (2):332-343.

Gerberick, G. F., J. D. Vassallo, L. M. Foertsch, B. B. Price, J. G. Chaney, and J.-P. Lepoittevin. 2007. Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. *Toxicological Sciences* 97 (2):417-427.

Gober, M. D., and A. A. Gaspari. 2008. Allergic contact dermatitis. In *Dermatologic Immunity*: Karger Publishers. p. 1-26.

Johnson, W. 1987. FINAL REPORT ON THE SAFETY ASSESSMENT OF PANTHENOL AND PANTOTHENIC-ACID. *Journal of the American College of Toxicology* 6 (1):139-162.

Martin, S. F. 2012. Contact dermatitis: from pathomechanisms to immunotoxicology. *Experimental dermatology* 21 (5):382-389.

Nelson, J. L., and C. M. Mowad. 2010. Allergic contact dermatitis: patch testing beyond the TRUE test. *The Journal of clinical and aesthetic dermatology* 3 (10):36.

OECD. 442C. OECD Guideline for Testing Chemicals: *In Chemico* Skin Sensitization: Direct Peptide Reactivity Assay (DPRA).

Saint-Mezard, P., A. Rosieres, M. Krasteva, F. Berard, B. DUBOIS, D. KAISERLIAN, and J.-F. NICOLAS. 2004. Allergic contact dermatitis. *European Journal of Dermatology* 14 (5):284-295.

Stables, G., and S. Wilkinson. 1998. Allergic contact dermatitis due to panthenol. *Contact Dermatitis* 38 (4):236-237.

Thyssen, J., J. McFadden, and I. Kimber. 2014. The multiple factors affecting the association between atopic dermatitis and contact sensitization. *Allergy* 69 (1):28-36.