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IN VITRO SKIN IRRITATION STUDY

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Prepared by:

[redacted] Ph.D., DABT
Cardno ChemRisk – Supervising Health Scientist

[redacted] Ph.D.
Cardno ChemRisk – Managing Health Scientist

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1. INTRODUCTION

Cardno ChemRisk was asked by WEN By Chaz Dean ("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 engaged in several tests to assess the skin irritation and sensitization potential of the WEN Products, which, according to a review of the scientific literature, can lead to hair loss in some individuals. One such test we performed on the WEN Products was an *in vitro* irritation test to evaluate the skin irritation potential of the WEN Products and certain competitor products.

We utilized the Organisation for Economic Co-operation and Development (OECD) 439 *in vitro* irritation testing guideline to evaluate the skin irritation potential of WCD's cleansing conditioners and competitor products. 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 Test 439 Describes an in vitro procedure that may be used for the hazard identification of irritant chemicals based on reactivity to a reconstructed human epidermis. This guideline test utilizes a three-dimensional reconstructed human epidermis cultured *in vitro*, to evaluate the "initial step of the inflammatory cascade/mechanism of action (cell and tissue damage resulting in localized trauma) that occurs during irritation *in vivo*" (OECD 439). The test has been validated by the OECD to determine the skin irritancy potential of substances either as a stand-alone replacement test or as a partial replacement test within a testing strategy for *in vivo* skin irritation testing (OECD 439). In addition, the OECD 439 test may be used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with the European Union (EU) classification and the United Nations (UN) Globally Harmonized System of Classification (GHS) (OECD 439).

2. BACKGROUND

2.1 Skin Irritation

In animal experiments, dermal irritation has been defined as "the production of reversible damage of the skin following the application of a test substance for up to 4 hours" (OECD 439). Erythema (redness), eschar (scabs) and edema (swelling) are common manifestations of dermal irritation (Gallegos Saliner, Tsakovska et al. 2007). At times, additional symptoms include alopecia, hyperkeratosis, hyperplasia, and scaling. The standard test for evaluating skin irritation is the Draize rabbit dermal irritation test (Gallegos Saliner, Tsakovska et al. 2007). However, in light of recent efforts by the OECD to reduce and replace animal testing, the reconstructed human epidermis test (OECD 439) has been validated as a stand-alone replacement test or as a partial replacement test within a testing strategy for *in vivo* dermal irritation testing (OECD 439; OECD 2009). 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

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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.

3. METHODOLOGY

Reconstructed human epidermis tissue samples were treated in triplicate with test articles (WCD Sweet Almond Mint Cleansing Conditioner, WCD Lavender Cleansing Conditioner, WCD Pomegranate Cleansing Conditioner, [redacted] a negative control (phosphate buffered saline), and a positive control (5% sodium dodecyl sulfate) for 60 minutes. After 60 minutes of treatment, test articles and controls were washed off the tissue and the tissue was incubated for 24 hours. Following treatment and incubation, the viability of the tissues was determined using methyl thiazole tetrazolium (MTT) uptake and reduction assay. Tissue viability was reported as a percent of negative control values.

Skin irritation classification was based on the classification criteria illustrated in Table 1. Briefly, a mean tissue viability of 50% or less classifies the test article as an irritant; a mean tissue viability of more than 50% classifies the test article as a non-irritant.

Table 1. Skin Irritation Test – Classification Criteria

Mean Tissue % Viability (% of Negative Control)	Classification	
	EU	GHS
Mean tissue viability ≤ 50%	Irritant	Category 2
Mean tissue viability > 50%	Non-irritant	No category

4. RESULTS AND DISCUSSION

Two controls and six test articles (3 WEN Products and 3 other commercially available products) were evaluated for skin irritation potential. The skin irritation test results are summarized in Table 2. Briefly, the positive control reduced the mean tissue viability below 50% and was classified as a Category 2 Irritant. Tissue treated with the negative control and test articles WEN Sweet Almond Mint Cleansing Conditioner, WEN Lavender Cleansing Conditioner, WEN Pomegranate

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Cleansing Conditioner, [redacted] had mean tissue viabilities above 50% and were classified as non-irritants.

Table 2. Skin Irritation Test Results

Sample	Mean Tissue Viability (%)	Classification	GHS Category
Negative Control (Phosphate Buffered Saline)	100.0	Non-Irritant	No category
Positive Control (5% Sodium Dodecyl Sulfate)	2.7	Irritant	Category 2
WCD Sweet Almond Mint Cleansing Conditioner	96.7	Non-Irritant	No category
WCD Lavender Cleansing Conditioner	103.3	Non-Irritant	No category
WCD Pomegranate Cleansing Conditioner	98.7	Non-Irritant	No category
[redacted]	116.5	Non-Irritant	No category
[redacted]	114.0	Non-Irritant	No category
[redacted]	108.2	Non-Irritant	No category

5. CONCLUSION

Cardno ChemRisk performed an *in vitro* skin irritation study on three WEN Products (Sweet Almond Mint, Lavender, and Pomegranate Cleansing Conditioners) and three additional commercially available cleansing conditioners [redacted] The results of the study showed that all test articles did not illicit an irritation response *in vitro*. According to the OECD, these results are a reliable prediction of a substance's irritation classification *in vivo*. Therefore, the use of the WEN Products and the other commercially available cleansing conditioners tested, would not be expected to cause dermal irritation in consumers using these products.

6. REFERENCES

Gallegos Saliner, A., I. Tsakovska, et al. (2007). "Evaluation of SARs for the prediction of skin irritation/corrosion potential–structural inclusion rules in the BfR decision support system." SAR and QSAR in Environmental Research **18**(3-4): 331-342.

OECD (439). "OECD Guidelines for the Testing of Chemicals: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method."

OECD (2009). "Chemical Safety and Animal Welfare."