[redacted]

IN VITRO PHOTOTOXICITY STUDY

[redacted]

Prepared by:

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

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

November 30, 2019

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, through a search of the scientific and/or medical literature, we identified potential causes of hair loss and/or any other adverse dermal event, and then tested the WEN Products to determine whether such potential cause could have been induced by use of the Products. One such potential cause for hair loss identified in the literature that is the subject of this report was phototoxicity. In order to evaluate the phototoxic potential of the WEN Products, we performed *in vitro* phototoxicity testing on three of the best-selling and most complained about of the WEN Products (Sweet Almond Mint, Lavender, and Pomegranate).

Cardno ChemRisk utilized the Organisation for Economic Co-operation and Development (OECD) 432 *in vitro* 3T3 NRU phototoxicity test to evaluate the phototoxicity potential of WCD's cleansing conditioners. 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 OECD 432 guideline, which is a test that utilizes cells cultured *in vitro* to compare cytotoxicity of a chemical or substance in the presence and absence of irradiation exposure to a non-cytotoxic dose of simulated solar light (OECD 432). The reliability and relevance of the tests has been evaluated by the OECD and determined to be predictive of acute photoxicity effects in animals and humans *in vivo*.

2. BACKGROUND

Phototoxicity is defined "as a toxic response from a substance applied to the body which is either elicited or increased … after subsequent exposure to light" (OECD 432). Adverse reactions resulting from phototoxicity may include skin irritation in form of redness or blistering. A common feature of phototoxic chemicals or substance is the ability to absorb energy from light within the range of sunlight. OECD 432 guidelines recommend that prior to *in vitro* evaluation, the UV/VIS absorption spectrum of the test article be determined. Those articles that exhibit no or little absorption are unlikely to be photoreactive (OECD 432). The *in vitro* 3T3 Neural red uptake (NRU) phototoxicity test evaluates the cytotoxicity of a chemical in the presence versus absence of light. Further, the *in vitro* 3T3 NRU phototoxicity test is considered an accurate predictor of acute phototoxicity *in vivo (OECD 432).*

3. METHODOLOGY

[redacted]

Prior to initiation of *in vitro* studies, test articles (WCD Sweet Almond Mint Cleansing Conditioner, WCD Lavender Cleansing Conditioner, WCD Pomegranate Cleansing Conditioner) were evaluated using UV/VIS spectral scan encompassing the wavelengths 200 to 900 nm to determine ability to absorb light.

Baclb/c 3T3 mouse embryonic fibroblast cells were treated in duplicate 96-well microplates with test articles (Sweet Almond Mint Cleansing Conditioner, Lavender Cleansing Conditioner, Pomegranate Cleansing Conditioner), and a positive control (chlorpromazine; CPZ) for 60 minutes. Test articles were prepared in a concentration range using serial dilutions in Hank's buffered saline solution (HBSS). After 60 minutes, one plate of treated 3T3 cells was irradiated with one dose of 5 J/cm^2 Solar Simulated Light (SSL), containing wavelengths of UVA and visible light with more than 99% of UVB blocked to simulate sunlight. The duplicate plate of treated 3T3cells remained in the dark. Following UV irradiation, 3T3 cells were washed and incubated with culture medium for 24 hrs, at which time cell viability was determined by neural red uptake over three hours. Cell viability was reported as the EC_{50} value, or concentration at which cell viability was reduced by 50%.

The acceptance criteria for the OECD 432 guideline was based upon neural red uptake in 3T3 cells following treatment with positive control, CPZ, in the presence (+SSL) and absence (No SSL) of irradiation. The test meets acceptance criteria if it meets the following:

1. $EC_{50\ No\ SSL}$ is within the range of 7.0 – 90.0 µg/ml;
2. $EC_{50\ +\ SSL}$ is within the range of 0.1 – 2.0 µg/ml; and
3. Photo-Irritant Factor (PIF = $EC_{50\ No\ SSL}$ / $EC_{50\ +\ SSL}$) is greater than or equal to 6.0

Further, according to the test guidelines, a test article is considered to have a positive effect (i.e. phototoxic potential, if the calculated PIF is greater than 5.0

4. RESULTS AND DISCUSSION

Three test articles (Sweet Almond Mint, Lavender, and Pomegranate) and one positive control (CPZ) were evaluated for phototoxicity potential. The *in vitro* test results are summarized in Table 1. Briefly, the positive control (CPZ) reduced cell viability with a PIF of 55.3, consistent with the acceptance criteria for OECD 432 and its known phototoxic potential. Sweet Almond Mint Cleansing Conditioner, Lavender Cleansing Conditioner, and Pomegranate Cleansing Conditioner did not exhibit any phototoxic potential, as EC50 values were greater than 0.1% (the maximum tested concentration) and the PIF could be not calculated. These results demonstrate that the WEN Products evaluated in this study do not exhibit phototoxicity.

Table 1. Phototoxicity Test Results

Test Sample	Concentration Range	EC_{50} No SSL	EC_{50} +SSL	PIF
WCD Sweet Almond Mint Cleansing Conditioner	0.007 – 0.1%	> 0.1%	> 0.1%	n/a*
WCD Lavender Cleansing Conditioner	0.007 – 0.1%	> 0.1%	> 0.1%	n/a*
WCD Pomegranate Cleansing Conditioner	0.007 – 0.1%	> 0.1%	> 0.1%	n/a*
Positive Control (CPZ)	No SSL: 6.81 – 100 µg/ml +SSL: 0.22 – 3.16 µg/ml	22.1 µg/ml	0.4 µg/ml	55.3

*PIF could not be calculated since EC_{50} values for both No SSL and +SSL results were > 0.1%.

5. CONCLUSION

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

6. REFERENCES

OECD 432. OECD Guideline for Testing of Chemicals: *In Vitro* 3T3 NRU phototoxicity test. April 2004.