Hair Pollution Protection Assay Analysis

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Tradename: Phytofuse Renew® SF

Code: I16586B2

CAS #: 90106-73-3

Test Request Form #: 7750

Lot #: N200623C

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Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Hair Pollution Protection Assay

Introduction

The deleterious effects of pollution in skin and hair care has become a new frontier for anti-aging active ingredients. Environmental pollutants are results of automobile exhaust gas, industrial emissions, and even emissions from simple household chores such as cooking and cleaning. Hair is subject to these environmental aggressions as well as UV irradiation and, unlike the skin, hair is quite vulnerable and lacks self-protection mechanisms. Exposure to environmental pollution can result in dry, brittle hair with decreased strength and elasticity.

Our hair pollution protection assay was conducted to assess the ability of **Phytofuse Renew® SF** to protect the hair from the oxidative effects of air pollution. Hair swatches were treated and exposed to cigarette smoke, and peroxidation of hair lipids were assessed using a Malonaldehyde (MDA) Assay. Pollutant cigarette smoke is a suitable substance containing all key pollution components such as reactive oxygen species (ROS), reactive nitrogen species, and electrophilic aldehydes. Reactive oxidants as well as free radicals from cigarette smoke are closely associated with oxidative stress and secondary oxidative events, such as lipid peroxidation.

The Malondialdehyde (MDA) assay is useful for quantitatively measuring the end product of lipid peroxidation and determining oxidative stress. MDA is frequently used as a bio marker for oxidative stress and, in this case, lipid peroxidation (the breaking down of lipids) due to environmental stress. An increase in MDA indicates an increase in lipid peroxidation and oxidative stress.

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Materials & Methods

Testing was performed on sixteen total virgin hair swatches. A testing group consisted of four swatches, each of a different ethnicity: European, Afro-American, Asian, and Indian. Four testing groups were observed: untreated, shampoo/conditioner, saturation with rinse, and saturation without rinse. For the shampoo/conditioner testing group, the hair swatches were washed with both a generic shampoo and conditioner formulation incorporating 2.0% **Phytofuse Renew® SF**. For the saturation with rinse testing group, the hair swatches were fully saturated in a 2.0% **Phytofuse Renew® SF** in water solution, allowed to completely air dry, rinsed with 500 grams of deionized water, and allowed to air dry again. For the saturation without rinse testing group, the hair swatches were fully saturated in a 2.0% **Phytofuse Renew® SF** in water solution and allowed to completely air dry.

Testing was performed in a custom 22”x15”x6” smoke chamber. A standard filter pump was placed in the side of the chamber as air supply and standard ceramic filter funnel was placed in the top of the chamber for continuous exposure. Each testing group of hair was hung together in designated 3x3 inch section at the top of the smoke chamber. For stagnant exposure testing, four standard 2 ounce glass retain jars were placed inside the chamber.

One hundred cigarettes were placed into the ceramic filter funnel, with the tip of the cigarettes facing outward. Cigarettes were Riverside brand and all of the cigarette filters were torn off at the base. The cigarettes stood upright tightly packed in the filter funnel and the tips were torched. The hair samples were in the smoke chamber for a total of thirty minutes. For the first ten minutes, continuous air was pulled through the filter pump system and the cigarettes were re-torched when needed. The final twenty minutes, the hair samples underwent stagnant exposure. During stagnant exposure, five cigarettes were placed standing in each of the four retain jars and positioned at each corner of the smoke chamber and the tips of the cigarettes were lit every five minutes. During stagnant exposure, the smoke chamber was fully sealed with the exception of small opening in the top where the original filter funnel was to allow oxygen into the system with minimal smoke loss. After the hair samples completed the time in the smoke chamber, each individual hair sample was placed in a sealed plastic bag for testing.

Hair lipid peroxidation was assessed with the Abcam Lipid Peroxidation (MDA) Assay Kit (ab118970). Samples were taken from the middle portion of the hair strand, totaling 0.2g of tissue weight. To prepare the hair for testing, each hair sample was washed with cold phosphate buffer solution (PBS) and placed in 15ml conical tubes with the MDA lysis buffer. The pestle portion of a dounce grinder and vortex were used to agitate the hair and release the lipids.

The Lipid Peroxidation (MDA) Assay Kit (Colorimetric/Fluorometric) (ab118970) is a convenient tool for sensitive detection of the MDA present in a sample. The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically using optical density.

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Results

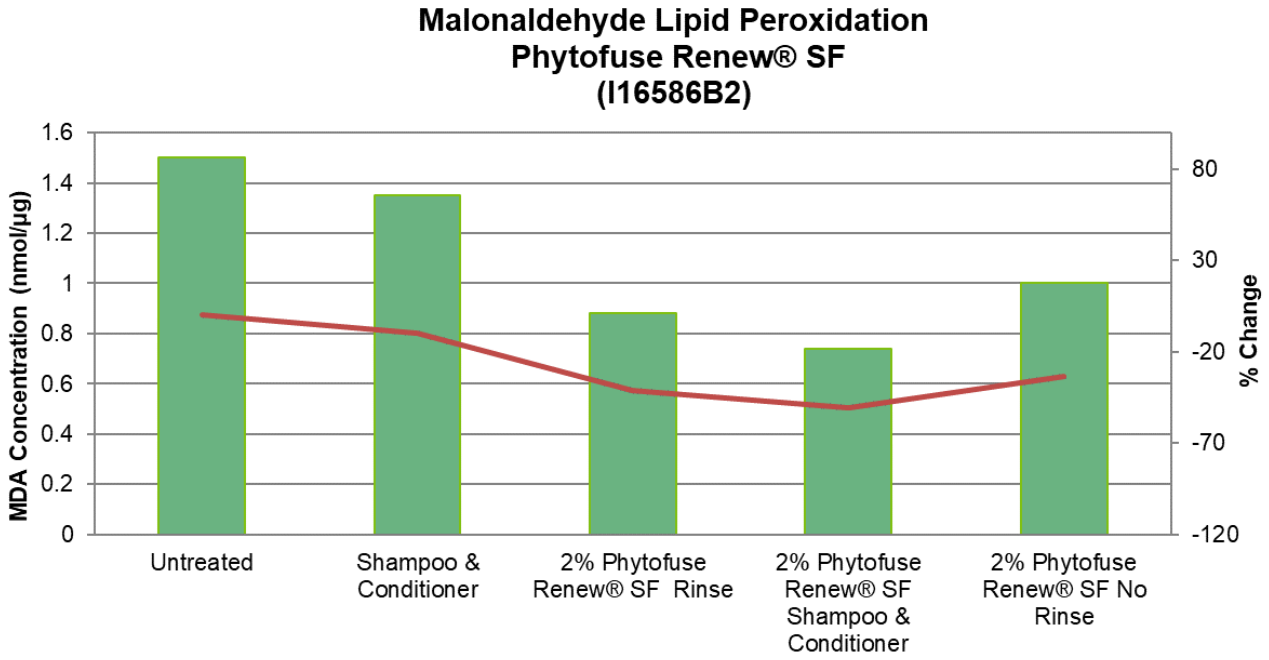

Figure 1. Average Values of MDA and Percent Change

Phytofuse Renew® SF at a concentration of 2.0% demonstrated significantly lower levels of MDA than the control standards. Hair samples treated with 2.0% **Phytofuse Renew® SF** in Shampoo and Conditioner showed 41.8% less MDA than the hair samples treated with the control shampoo and conditioner. Hair samples saturated in 2.0% **Phytofuse Renew® SF** with Rinse showed 69.0% less MDA than the untreated hair sample. Hair samples saturated in 2.0% **Phytofuse Renew® SF** without Rinse showed 41.9% less MDA than the untreated hair sample.

Discussion

The pollutant cigarette smoke contains key pollution components such as reactive oxygen species, reactive nitrogen species, and electrophilic aldehydes. Reactive oxidants as well as free radicals from cigarette smoke are closely associated with oxidative stress and secondary oxidative events, such as lipid peroxidation. Lipid peroxidation refers to the oxidative degradation of lipids. In this process, free radicals take electrons from the lipids, resulting in cell damage. Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. Lipid peroxidation forms reactive aldehydes such as MDA as natural byproducts. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage.

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An interesting observation from the results of this study showed that hair samples saturated in 2.0% **Phytofuse Renew® SF** without Rinse showed 41.9% less MDA than the untreated hair sample while hair samples saturated in 2.0% **Phytofuse Renew® SF** with Rinse showed 69.0% less MDA than the untreated hair samples. We hypothesize the apparent increase in pollution protection after rinsing the product from the hair samples to be due to the removal of hair surface lipids during rinse. Surface lipids are naturally removed during the rinsing process, lowering the number of lipids available to react with pollutants. A decreased amount of lipids present corresponds to a decreased production of MDA.

In this study, **Phytofuse Renew® SF** was tested to evaluate its effects on the inhibition of lipid peroxidation of hair samples exposed to air pollution. At a concentration of 2.0%, **Phytofuse Renew® SF** demonstrated significantly lower levels of MDA than the untreated controls. It can therefore be concluded that at normal use concentrations **Phytofuse Renew® SF** can be used as a hair pollution protection active ingredient.