Airborne Pollution Hair Protection Assay

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Tradename: Phytofuse Renew®

Code: 16586

CAS #: 90106-73-3

Test Request Form #: 12423

Lot #: 9404125

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Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Airborne Pollution Hair Protection Assay

Introduction

Hair lacks self-protection mechanisms and is vulnerable to airborne pollutants as they easily penetrate hair fibers and oxidize proteins, leaving it dry, brittle, and damaged. Hair proteins play a key role in structural integrity as the physical bonds between amino acids provide mechanical stability and chemical resistance to hair fiber. Tryptophan, a sensitive amino acid and chromophore, degrades when exposed to environmental stressors such as pollutants and is utilized as a proxy for hair damage. This abundant protein has a well-defined spectral range, so as tryptophan oxidizes in response to pollution exposure, relative fluorescence decreases, indicating hair shaft damage.

Given the increasing amount of global pollution, cosmetic products offering hair protection from airborne pollutants are a critical component in limiting external factors detrimental to hair health and appearance. These cosmetic products combat air pollution by preventing pollutant deposition while promoting hair health.

Accordingly, an *ex vivo* airborne pollution hair protection assay was conducted to assess the ability of **Phytofuse Renew®** to protect hair from airborne pollutants.

Assay Principle

Air pollution has many constituents responsible for its damaging effects including oxides, ozone, and particulate matter (PM). The level of PM 2.5 is often used to gauge environmental pollution where concentrations below 50 ug/m^3 are considered good air quality and those above 500 ug/m^3 are considered hazardous conditions. One cigarette can produce between 600-1000 ug/m^3 of PM 2.5 making them a good proxy for severe pollution exposure. PM 2.5 easily penetrates hair fibers and damages hair via protein oxidation. Therefore, cigarette smoke was utilized as a model for airborne pollutants as exposure can be standardized without heavy influence of environmental factors. Human hair tresses were treated with test materials and exposed to cigarette smoke in a controlled chamber. Tryptophan was extracted from hair tresses and quantified using fluorescence as a proxy for hair fiber damage, with decreases indicative of more damage.

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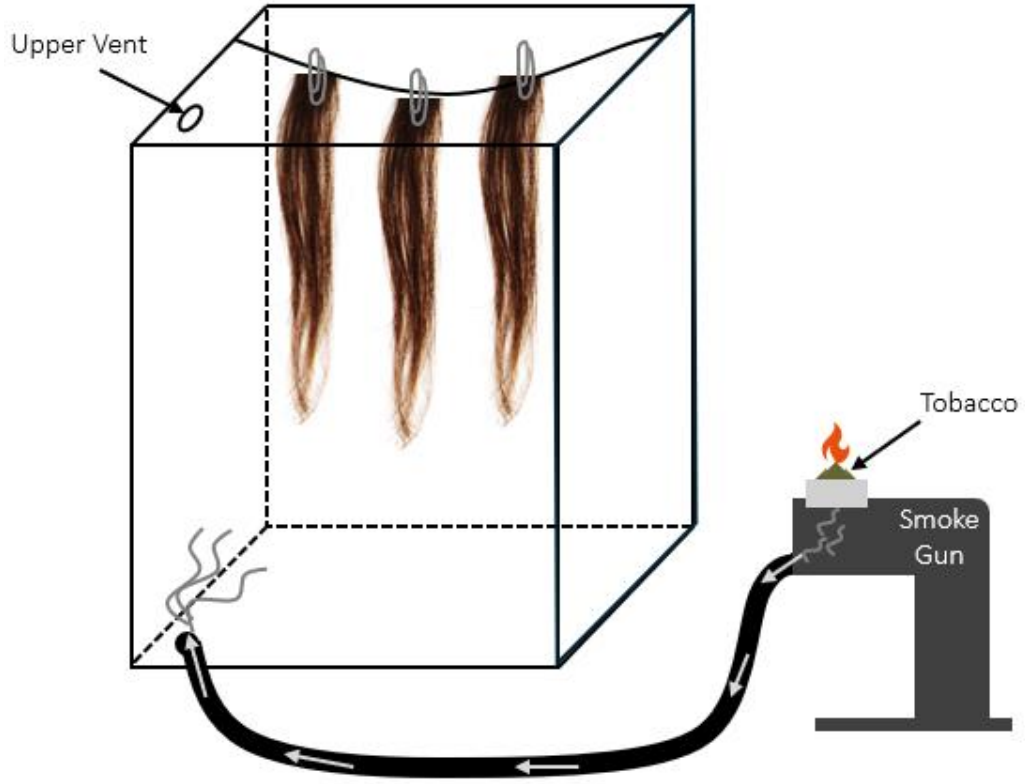

Image 1. Smoke Exposure Chamber. Cigarette tobacco is burned, and the smoke is pumped into the chamber.

Materials

A. Hair Samples: Human Virgin Brunette Hair Tresses
B. Equipment: Synergy HT Microplate Reader; Pipettes; Electric Smoke Gun; Seneca Full Flavor American Blend No Filter King Size Cigarettes*; Smoke Exposure Chamber
C. Reagents: Sodium Hydroxide (2 M)
D. Products: Base Shampoo (Table 1)
E. Culture Plate: Flat Bottom 96-Well Black Side/Clear Bottom Microplate*
F. Software: Excel Analysis ToolPak (Microsoft)
G. Other: Sterile disposable pipette tips; 15 mL test tubes; 50 mL test tubes; Filters
*Or suitable alternatives, subject to change without notice based off vendor availability

Table 1. Base Shampoo Compositional Breakdown.

Base Shampoo Formulation	
INCI	**%**
Water	41.0
Guar Hydroxypropyltrimonium Chloride	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0
Cocamidopropyl Betaine	15.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0
Polysorbate 20	2.0
Fragrance	2.0

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Methods

Three virgin brunette hair tresses were collected and treated with water (Cigarette Smoke Control), 2.0% **Phytofuse Renew®** in water, or left as an Untreated Control (no product). Each tress was fully saturated in its respective treatment and excess product was towel dried off. After treatment, tresses were airdried then placed in the smoke exposure chamber ensuring each tress hung naturally and was not in contact with other tresses or the chamber walls. The Untreated Control was not placed in the smoke chamber.

Cigarette smoke was pumped into the smoke chamber until all tobacco was exhausted. The hair tresses were exposed to the cigarette smoke for one hour with a stagnant smoke concentration of two cigarettes/nine liters, or > 1000 ug/m^3 PM 2.5. After one hour of stagnant exposure, smoke was slowly released from the chamber for two hours before the hair tresses were removed from the chamber. Next, all three tresses were shampooed once to remove any residues deposited on the fiber surface and allowed to airdry.

Tryptophan was extracted from each hair tress by dissolving the hair in a 2 M sodium hydroxide (NaOH) solution (1:1 Hair:NaOH). After sitting for 24 hours, the solutions were filtered and 200 µL of each extract was added in duplicate to a 96 well black side/clear bottom plate. Fluorescence was measured at an excitation wavelength of 290 nm and an emission wavelength of 355 nm. Tryptophan degradation was evaluated based on changes in relative fluorescence compared to the Untreated Control.

Three separate experiments were performed with conditions in duplicate and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. Percent change in tryptophan for each treatment group was calculated as follows:

$$Percent\ Change\ (\%) = \left(\frac{(Fluorescence_{Treatment} - Fluorescence_{Untreated\ Control})}{Fluorescence_{Untreated\ Control}}\right) \times 100$$

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Results

The data obtained met criteria for a valid assay as the controls performed as anticipated. The Cigarette Smoke Control exhibited reductions in tryptophan after smoke exposure while the tresses treated with **Phytofuse Renew®** at 2.0% demonstrated minimal changes in tryptophan after exposure.

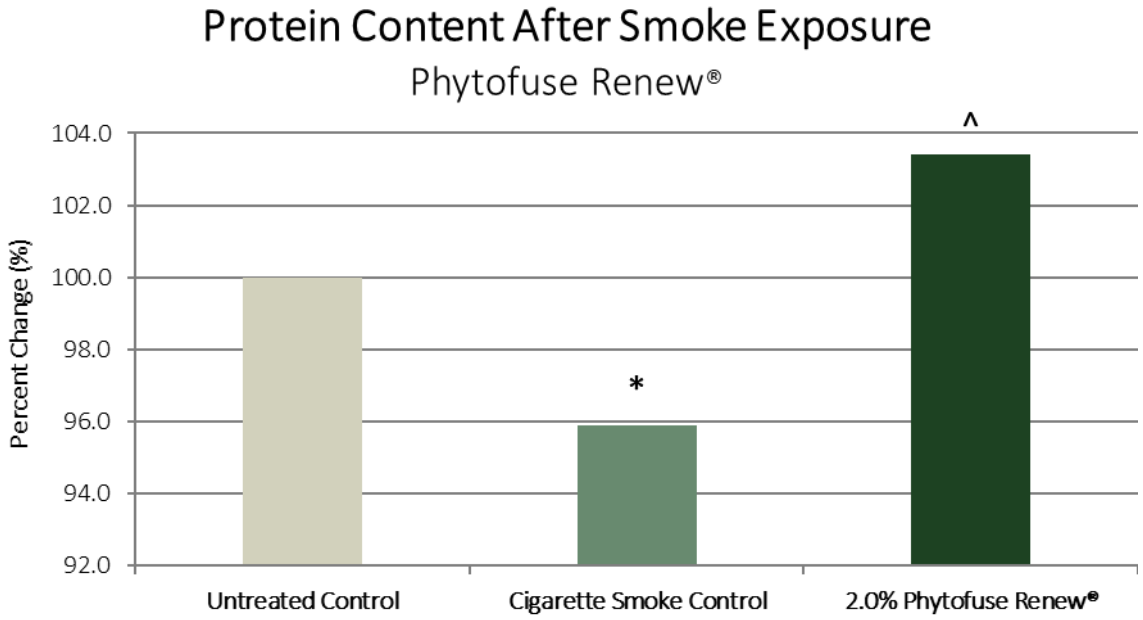

Figure 1. Percent Change in Tryptophan after Smoke Exposure Relative to Untreated Control Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) between the two conditions.

Table 2. Results from one-way ANOVA Statistical Analysis of Tryptophan Degradation between the Untreated Control and Smoke Exposed Hair. * indicates significance ($p \leq 0.05$).

	Cigarette Smoke Control	2.0% **Phytofuse Renew®**
P-value	0.022*	> 0.05

Table 3. Results from one-way ANOVA Statistical Analysis of Tryptophan Degradation after Exposure between the two conditions. ^ indicates significance ($p \leq 0.05$).

	Cigarette Smoke Control vs 2.0% **Phytofuse Renew®**
P-value	0.026^

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Discussion

As shown in Figure 1, the Cigarette Smoke Control hair tresses exhibited a 4.1% reduction in tryptophan content compared to the Untreated Control (Table 2). These data demonstrate the detrimental effects of airborne pollutants on hair proteins.

Alternatively, hair tresses treated with 2.0% **Phytofuse Renew®** demonstrated a 3.4% increase in tryptophan compared to the Untreated Control (Figure 1, Table 2). Furthermore, 2.0% **Phytofuse Renew®**-treated hair tresses demonstrated 7.5% more tryptophan compared to the Cigarette Smoke Control hair tresses (Figure 1, Table 3). These data demonstrate **Phytofuse Renew®** attenuates tryptophan degradation in hair under highly polluted conditions.

Collectively, these results demonstrate **Phytofuse Renew®** blunts airborne pollutant-induced hair protein degradation, thereby protecting hair fibers from damage. Taken together, these data indicate **Phytofuse Renew®** promotes hair health by exerting anti-pollution properties on hair when used at the recommended use-levels.