National Pollen and
Aerobiology Research Unit
at the University of Worcester

**CONFIDENTIAL REPORT**

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**FOR HAYMAX LTD**

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HayMax is marketed as a product made from beeswax and sunflower oil that prevents some pollen from entering the body through the nose. Pollen grains are trapped as they enter the nostrils making the nose more efficient as a natural filter.

This product is readily available over the counter in outlets in the UK, online and in some international countries.

A small study was previously conducted in 2009 which aimed to assess the number of pollen grains that are trapped by the Haymax balm when applied in a standard manner to the rim and the inside of nostrils compared with the amount deposited on uncoated nostrils. The study did not assess the impact of HayMax on symptoms of hay fever.

The 2009 study concluded that there was a significant difference at the $p \leq 0.05$ level by the t test between the results for grass pollen grains between uncoated nostrils and those with Haymax. Similarly there was a significant difference at the $p \leq 0.01$ level by the t test for "other " pollen grains between uncoated nostrils and those with Haymax. There was also a significant difference at the $p \leq 0.05$ level by the t test for total pollen grains between uncoated nostrils and those with Haymax.

This further study looked at whether HayMax can trap indoor and outdoor airborne particles such as pollens in a more controlled environment.

The study required thirty healthy volunteers who did not suffer from allergic rhinitis to visit NPARU 4 times over a 3 week period. Thirty five people were initially signed up with an expected drop out rate of 14% (5 participants). Volunteers with Asthma or any nasal obstruction were excluded.

**Indoor allergens**

The most common triggers of indoor allergy symptoms are house dust mite (HDM) droppings, animal dander, cockroach droppings and moulds.

The major house dust mite allergen (Der P 1) and cat dander allergen (Fel d 1) were selected as these are common allergens and participants would have been exposed to them previously even if they don't have a cat themselves. Studies have shown surprisingly high levels of cat allergen are found within places of work.

House dust mite allergen (HDM) represents one of the most frequent and potent allergen sources and Der P 1 is the principal HDM allergen. Research has shown that more that 50% of allergic patients and more than 80% of asthmatic children are sensitized to mite allergens

In general the threshold level of exposure to HDM allergen for allergic sensitization is considered to be 2µg/g dust and the level for asthma in sensitized individuals is

considered to be 10µg/g dust. This translates into 2mg of purified Der p 1 house dust mite allergen extract.

The incidence of allergic symptoms to pet dander has increased over recent years as a consequence of lifestyle changes that have enhanced ambient exposure to pet allergens. Fel d 1 is produced largely by cat saliva and sebaceous glands and is the primary allergen present on cats.

For cat allergen the levels considered are 1 µg/g dust for sensitization and 8µg/g dust for allergic reaction in sensitised individuals. This translates into 1mg of purified Fel d 1 cat allergen extract.

**Pollens**

Grass pollen is the most important allergenic pollen type in the UK as research indicates that 95% of people with seasonal allergic rhinitis are allergic to this group. Within the allergic rhinitis group 25% of sufferers are allergic to tree pollen and 20% are allergic to weed pollen.

Grass and tree pollens were selected as these effect a higher percentage of the population who suffer from pollen allergy.

Assessments using the weight of pollen grains were made to calculate the amount of pollen that was needed to be released into the chamber to replicate a high pollen count.

Fungal spores and ragweed pollen were also considered. Although fungal spores occur naturally in the environment particularly in the autumn without effect on humans, they can cause a number of conditions with varying consequences. Some fungal species are primary pathogens, invading the body through the respiratory tract and others can act as a secondary pathogen, invading in circumstances where the host is predisposed to infection.

Ragweed is a rare plant in the UK and is only found in a few sites in the southern part of the country. It has highly allergenic pollen and so was rejected as its inclusion may have caused severe reactions in participants whose systems would not have encountered it in any quantity before.

**Environmentally controlled test chamber**

NPARU has an environmentally controlled test chamber which is capable of recreating most climate types and environmental conditions worldwide. The chamber was set to an ambient temperature of $18^0$C .

Within the test chamber is a self-contained allergen challenge chamber which can be used to replicate any pollen levels at any time of the year and to avoid all problems associated with unpredictable pollen seasons. It can also be used to replicate conditions that would be found in the average household in terms of indoor allergens.

On very high pollen count days, pollen counts can reach between 250 and 400 pollen grains per cubic metre. Preliminary work on pollen counts established how many pollen grains need to be released into the air to replicate high pollen count days.

A series of strategically placed fans create air flow which keeps the allergen airborne and maintains the allergen levels during the period that participants are in the chamber. The inside of the chamber has been coated with an anti-static product to prevent allergens from being attracted to and sticking to the walls via static charge.

The timing of visits from participants was organised such that no allergen would still be airborne from a previous visit. The chamber was cleaned down between visits.

**Treatment blinding**

All researchers except the principal investigator and all participants were blinded to the name of the product being used and in which nostril the product was applied.

**Randomisation**

A random number generator was used to determine which nostril was used for each visit of a participant.

**Nasal Air flow**

The airflow through the nasal passage is normally asymmetrical, with one nasal passage having the dominant airflow. This asymmetry of nasal airflow is not fixed, as the dominant airflow spontaneously alternates from one nasal passage to the other over a period of several hours. People are not normally aware of the alternation in nasal airflow between the two nasal passages as the total nasal resistance remains relatively constant because of the reciprocal relationship between the two sides of the nose.

The asymmetry in nasal airflow tends to be exaggerated when there is nasal infection or when lying down.

The effect of nasal airflow was physically minimised by excluding anyone with nasal obstructions, colds etc. and statistically minimised by the number of participants recruited. As symptoms were not being provoked in participants, nasal air flow was not affected during the visit time by nostrils becoming blocked etc.

**Applying HayMax**

The protocol for the application method was developed during the study conducted in 2009.

For each person the procedures were explained and nostrils were blown and wiped.

A standard amount of neutral (unscented) HayMax balm was applied evenly to the lower part and rim of one nostril using a cotton bud. The nostril to be used was pre-determined for each participant. HayMax was applied to the cotton bud before the participants' arrival so they were unaware of what product was being used and was applied by a researcher to ensure standard and even application.

**Allergen challenge chamber**

Before entering the chamber, each participant was required to put on protective clothing (lab coat, hair net, shoe protectors). This was to help prevent allergens from escaping from the chamber into the immediate environment on participant's clothing and also to protect anyone who subsequently came into contact with the participants after a visit and who suffered from allergies.

The participants then entered the chamber for 15 minutes accompanied by a researcher. They were instructed by the researcher to keep their mouths closed and not to talk during the entire time they were in the chamber. They were instructed to breathe normally through their noses. They remained standing throughout but were allowed to walk round if this was more comfortable than standing still.

The researcher acted as a control and during the outdoor allergen visits wore personal nasal samplers as well as the same protective clothing. They also breathed through their noses only while in the chamber. The researcher switched on the fans, set the timer and released the allergen being used for that visit.

**Allergen release**

Allergen levels of 1mg purified cat dander and 2mg purified House Dust Mite were weighed out into an inert lactose carrier to aid dispersion within the chamber. Pollen levels of 1.5mg were weighed out, no carrier was required to aid dispersion.

The relevant allergen for the visit was dispersed around the chamber using a central fan system.

After 15 minutes the fans were switched off and the participant and researcher left the allergen challenge chamber. Protective clothing was removed but left inside the Environmental test chamber.

The participant and researcher returned to the lab where the participants' nostrils were sampled.

**Sampling the nostrils for outdoor allergens**

The protocol for this was established by the previous study. Pollen was removed from the nostrils using a low tack tape which has been shown to remove any pollen captured by HayMax.

Each nostril was sampled separately onto a pre-labelled tape holder. The tape was then mounted onto a microscope slide, and viewed using a high power microscope. The number of pollen grains adhering to the tape was counted.

**Personal Nasal Samplers**

A researcher entered the chamber with each participant wearing personal nasal samplers. These are designed to fit snugly inside the nostrils and capture any airborne particle which has been inhaled through the nostrils by the wearer. The wearer can breathe normally while wearing them.

Samples collected were then analysed and used as controls and for comparison with data from the participants.

The protocol for using the samplers was developed by Associate Professor & Principal Research Fellow Euan Tovey at the University of Sydney, Australia.

**Sampling the nostrils for indoor allergens**

Following exposure for fifteen minutes samples were taken from the nostril coated with HayMax using a cotton bud. The tip of the cotton bud was subsequently removed and immersed in 1ml PBS-T with a tungsten bead in 2ml centrifuge tubes.

**Wash out period**

Symptoms were not provoked in participants so a long washout period was not required. However a minimum of 24 hours was left between visits to ensure that no HayMax residue would remain from a previous visit.

**Processing the samples**

**Pollen samples**

The mounted slides were viewed individually under a microscope using x 40 magnification and pollen grains captured were counted. As the environment was controlled in the chamber i.e. only grass pollen grains were released during a 'grass pollen visit' and only tree pollen released during a 'tree pollen visit' grains could be counted with reliability that they had been correctly identified.

**Cat and house dust mite samples**

Following immersion in 1ml PBS-T with a tungsten bead in 2ml centrifuge tubes, samples were then run through a FASTPrep instrument (QBiogene, Irvine, CA) at a speed of 4.5 for 25s. Levels of airborne allergens (Fel d 1 and Der p 1) were detected from samples by application of 100µl aliquots to commercially available ELISA kits following the manufacturer's instructions (INDOOR biotechnologies Inc. Charlottesville, VA), but using 3,3',5,5' – Tetramethylbenzidine (TMB) to develop the assay instead of 2,2'-azino-di-(3 ethylbenzthiazoline sulphonic acid) (ABTS).
Colour development was halted after twenty minutes using 20µl 20% $H_2SO_4$ and optical density was read by at 450nm by an ELx800 Absorbance Microplate Reader (BioTek, Winooski, VT).

**Results**

No adverse effects were reported either during or after the study from the participants.

Statistical analysis was performed with the student t-test. P values ≤ 0.05 were considered significant.

**Indoor allergen**

A total of 34 people provided nasal samples for the indoor allergens which meant that there were 34 samples containing cat allergen (Fel d 1) and 34 samples containing house dust mite allergen (Der P1).

Participants were randomly assigned to groups, one group had HayMax applied to the left nostril and the other group had HayMax applied to the right nostril.

After the samples were processed, it was found that the mean level of Fel d 1 in the HayMax samples was 5132.32 pg $m^{-3}$ (to 2 decimal places))

This implies that HayMax has the capability of absorbing some cat allergen.

The mean level of Der p 1 in the HayMax samples was 4369.02 pg $m^{-3}$ (to 2 decimal places))

This implies that HayMax has the capability of absorbing some HDM allergen.

When the significance of the levels of Fel d 1 from samples taken from left nostrils coated in HayMax was compared to the levels of Fel d 1 from samples taken from right nostrils coated in HayMax was compared by a paired t-test there was no significant difference shown between the two groups (t = 1.461, df =14 , p value = 0.1661).

When the significance of the levels of Der p 1 from samples taken from left nostrils coated in HayMax was compared to the levels of Der p 1 from samples taken from right nostrils coated in HayMax was compared by a paired t-test there was no significant difference shown between the two groups (t = 0.3286, df =14 , p value = 0.7473).

**Outdoor allergens**

A total of 34 people provided nasal samples for the outdoor allergens (pollens) which meant that there were 34 samples of grass pollen and 34 samples of tree pollen. Each participant had a matching control sample provided by the accompanying researcher.

**Grass**

The mean number of grass pollen grains collected from nostrils was 618. The mean number of grass pollen grains collected in the personal nasal samplers worn by the researchers in the chamber was 1768. This means that on average HayMax captured the equivalent of 37% of the total of the control.

Analysis of the counts from each nostril for the product showed that there was no significant difference between whether the product was applied to the left or right nostrils.

The mean number of grass pollen grains collected from the left nostril coated with HayMax was 543 and from the right nostril coated with HayMax was 503. When the significance of this was tested by a paired t-test there was no significant difference shown between the two groups (t = 0.6344, df =16 , p value = 0.5348). (See Figure 1)

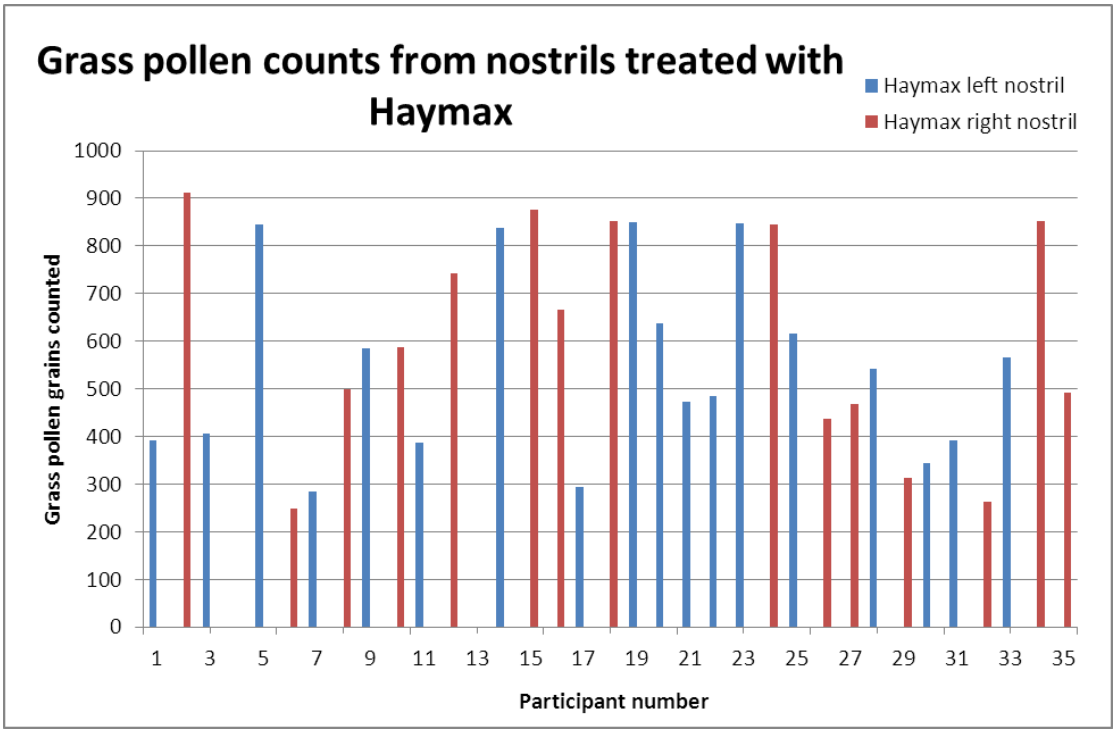


**Figure 1 Grass pollen grains collected by nostrils coated with HayMax**

**Tree**

The mean number of grass pollen grains collected from nostrils was 618. The mean number of tree pollen grains collected from nostrils was higher in the HayMax samples (634)

The mean number of tree pollen grains collected in the nasal samplers worn by the researcher in the chamber was 1847. This means that on average HayMax captured the equivalent of 34% of the total of the control,

Analysis of the counts from each nostril for the product showed that there was no significant difference between whether the product was applied to the left or right nostrils.

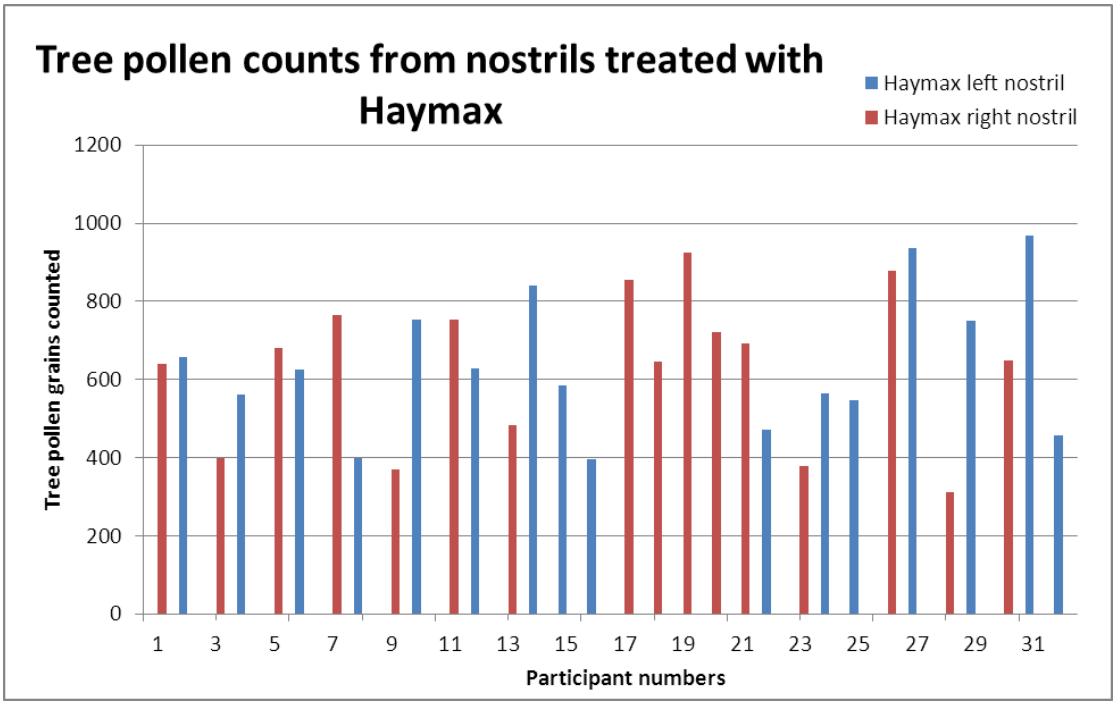


**Figure 2 Tree pollen grains collected by nostrils coated with HayMax**

**Discussion**

The nostrils and nasal cavities are part of the upper airway and serve as the main port of entry for the respiratory tract. They perform the function of scrubbing, conditioning and filtering inspired air. Some filtration is done mechanically by the thick hairs which grow inside the nostrils to help keep large particles from entering the nasal passages and by the hair like structures called cilia which line the surface of the trachea. Finer particles are more likely to evade these natural defences and reach the upper and lower respiratory tracts and this will include particles such as house dust mite allergen e.g. Der p 1, pollens e.g. grass pollen and cat allergen e.g. Fel d 1

The study in 2009 concluded that the results showed that the application of HayMax to the lower part and rim of the nostrils does trap significantly more pollen than an uncoated nostril. This would result in some reduction to the amount of allergen entering the nose. However the results do not give any indication of the amount of reduction of pollen entering the nose as many particles including pollen would go into the nose without being near to the surface of the nostrils. The study did not investigate the impact of Haymax on symptoms of hay fever and no inference in relation to symptoms can be made from the results.

This further study has shown that HayMax has the capability to trap indoor and outdoor airborne allergens and particles. It was also able to show that HayMax could block on average about a third of the pollen grains that were being captured in the personal nasal samplers worn by the researchers acting as controls.

This study did not investigate the impact of HayMax on symptoms of allergic rhinitis.

**References**

Day et al, The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper 2006 Clinical and Experimental Allergy Reviews, 6, 31–59

Eccles R., Role of the nasal cycle in respiratory defence Eur Respir 1996 9, 371-376

Morris D. O., Human allergy to environmental pet danders: a public health perspective Veterinary Dermatology (2010) 21, 441-449

Pittner et al, Component-resolved diagnosis of house dust mite allergy with purified natural and recombinant mite allergens Clin exp allergy (2004); 34: 597-603

Zhao et al, Effect of anatomy on human nasal air flow and odorant transport patterns: Implications for olfaction Chem Senses (2004,)29; 365-379

JAH Graham, PK Pavlicek, JK Sercombe, ML Xavier, ER Tovey The nasal air sampler: a device for sampling inhaled aeroallergens Annals of Allergy, Asthma & ImmunologyVolume 84, Issue 6, June 2000, Pages 599–604

Heinrich., J, Bedada, G.B., Zock, J-P., Chinn, S., Norba, D., Olivieri, M., Svanes, C., Ponzio, M., Verlato, G., Zillani, S., Jarvis, D., Luczynska, C. 2006. Cat allergen level: Its determinants and relationship to specific IgE to cat across European centers. *J Allergy Clin Immunol*. 118; 674-681

Boquete, M., Iraola, V., Fernández-Caldas, E., Arenas Villaroel, L., Carballada, FJ., González de la Cuesta, C., López-Rico, MR., Núñez Orjales,R., Parra,A., Soto-Mera, MT., Varela, S., Vidal, C. 2006. House Dust Mite Species and Allergen Levels in Galicia, Spain: a Cross- Sectional, Multicenter, Comparative Study. *J Investig Allergol Clin Immunol* 16(3): 169-176

Perfetti , L, Ferrari, M.,Galdia, E., Pozzia, V., Cotticac, D., Grignanid, E., Minoiac, C., Moscatoa, G. 2004. House dust mites (Der p 1, Der f 1), cat (Fel d 1) and cockroach (Bla g 2) allergens in indoor work-places (offices and archives). *Sci Total Environ* 328; 15–21

Raja, S., Xu Y., Ferro, AR., Jaques, PA., Hopke, PK. 2010. Resuspension of indoor aeroallergens and relationship to lung inflammation in asthmatic children. *Environ Int* 36; 8–14

Sakaguchi, M., Inouye, S., lrie, T., Miyazawa, H., Watanabe, M., Yasueda, H., Shida, T., Nitta, H., Chapman, MD. Schou, C., Aalberse, RC. 1993. Airborne cat (Fe/ d I), dog (Can f I), and mite (Der I and Der II) allergen levels in the homes of Japan
*J allergy clin immunol* 797-802

Lau, S., Schulz, G., Sommerfeld, C., Wahn U. 2001.Comparison of quantitative ELISA and semiquantitative Dustscreen2 for determination of Der p 1, Der f 1, and Fel d 1 in domestic dust samples. *Allergy* 56: 993–995

Swanson, MC., Campbell, AR., Klauck,MJ., Reed, CE. 1989 Correlations between levels of mite and cat allergens in settled and airborne dust *I Allergy Clin Immunol*; 776-783

Custovic, A., Simpson, A., Pahdi, H., Green, RM., Chapman, MD., Woodcock, A. 1998 Distribution, aerodynamic characteristics, and removal of the major cat allergen Fel d 1 in
British homes *Thorax* 53:33–38

Colloff, MJ. 2009. DUST MITES. CSIRO Publishing and Springer Science, Dordrecht, The Netherlands. 583 pp

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