UNIVERSITY OF SOUTHAMPTON

ELECTROSTATIC ALLERGEN CONTROL

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A thesis presented for the degree of
DOCTOR OF PHILOSOPHY

FACULTY OF ENGINEERING & APPLIED SCIENCE
DEPARTMENT OF ELECTRONICS & COMPUTER SCIENCE

This thesis was submitted for examination in July 2002.

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ABSTRACT

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The presence of allergens in the domestic environment has been linked to the development and elicitation of symptoms of atopic asthma. Although a number of techniques exist to reduce indoor allergens, attempts over the last thirty years have been largely unsuccessful. The objective of this present investigation was to determine whether corona discharge, the process used in air ionisers, could destroy the major household allergens of the Dermatophagoides house dust mite Der p1, Der f1, Der p2 and the major domestic cat allergen, Fel d1.

The method most commonly used to investigate the effects of a treatment on household allergens is to use a heterogeneous powder mixture, such as spent dust mite medium, containing the desired allergen. This method was improved upon by using evaporated aliquots of aqueous dust mite culture solution on aluminium foil. Using a simple point-to-plane corona apparatus of fixed inter-electrode distance and point radius, the effects of corona discharge were investigated on the allergens mentioned above. The results have shown that a specific number of allergen molecules are destroyed per unit exposure to corona discharge. This corona-destruction of allergens increased exponentially with both time and corona current until a plateau in the maximum percentage reduction in concentration was attained. Fel d1 was the most susceptible to destruction by negative corona, which led to a percentage reduction in concentration of 100% after 180 minutes at 25μA corona current. Dermatophagoides allergens experienced approximately similar reductions to each other. Fel d1, unlike the mite allergens tested, was not affected by positive corona. The effect of corona on Der p1 was not due to the corona product molecular ozone, or due to the presence of aluminium oxides on the planar electrodes. Der p1 was not destroyed under an atmosphere of 2.4% relative humidity using negative corona regimes, but was destroyed when subjected to positive corona under these conditions. The lack of renaturing after exposure to corona discharge and other results suggest that irreversible reactions occur between the corona products and the primary structure of the protein.

Chamber tests and room-scale exposures were performed, using an experimental ioniser and two commercially available ionisers, to determine whether corona discharge could translate well into a practical application for reducing allergen concentration in the domestic environment. The results of these tests have shown that Der p1 can be destroyed by up to 40.63% after one week's exposure to the experimental ioniser, 4m away from the corona source. Commercially available ionisers also destroyed Der p1 in both tests.

Thus, corona discharge has been shown to be an important and highly novel technique, which can be used to reduce the allergenic load from a number of sources in the domestic environment.

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PREFACE

This investigation into the effect of corona discharge on major domestic allergens is multidisciplinary and comprises a number of biological, biochemical and engineering topics. Firstly, *Chapter 1* provides the necessary background and review of literature needed to understand the concepts used in this work. Secondly in *Chapter 2*, preliminary experiments are described including the exposure of Der p1-carrying faecal particles to negative corona. The widely used method of using sieved, spent house dust mite medium to test the effect of a treatment on allergens was investigated for concentration homogeneity. This technique was improved and used to perform detailed investigations into the effect of corona discharge on allergens. These experiments, using a simple point-to-plane corona electrode arrangement, are described in *Chapter 3*. Furthermore, *Chapter 4* describes the chamber tests and room-scale exposures of Der p1 to an experimental and two commercially available ionisers. These tests were performed in order to determine whether the destruction of allergens by corona discharge could translate well into a practical application to reduce the levels of allergen in the domestic environment. Finally, *Chapter 5* draws conclusions from all the experiments and suggests possible future avenues for research. The *Appendix* gives a description of the various statistical terms and tests used.

ACKNOWLEDGEMENTS

I would like to thank the following people for their support and encouragement throughout my time at the Bioelectrostatics Research Centre: Professor John Hughes, Dr Lindsey Whitmore, Jonathon Noyce, Dr Karen Jerrim and Sabrina Higgins. I would also like to thank the *National Asthma Campaign* for funding this research. Finally, but not least of all, I would like to thank my family and fiancée, Aleksandra Djukic; without their constant enthusiasm and belief, this thesis would not have been written.

CHAPTER 1: INTRODUCTION

1.1 ASTHMA: EFFECTS AND GENERAL BACKGROUND

During the last three decades, many countries have experienced an increase in the prevalence and severity of asthma. Over the same period, the population in the developed world has spent more and more time indoors. Homes have become better insulated and energy efficient, resulting in a warm and humid environment with low ventilation rates. These conditions ideally suit house dust mite growth throughout the year (see *Section 1.4*).

Asthma is a syndrome in which there is recurrent reversible obstruction of the airways in response to stimuli that do not affect non-asthmatic subjects. The characteristic features of asthma are inflammatory changes in the airways associated with bronchial hyper-responsiveness (Bochner, Undem & Lichtenstein, 1994). Asthmatic airways respond to stimuli that may have little to no effect upon normal airways. These stimuli include emotional disturbances, respiratory tract infection, physical exercise, ingestion of drugs such as aspirin, inhalation of aerosols including fog, or chemicals such as ozone and sulphur dioxide, industrial chemicals such as toluene, and allergens in sensitised subjects including components of dust, pollen and animal dander (Morley, 1993).

Bronchial hyper-responsiveness leads to a distressing shortness of breath (dyspnoea) that reduces the quality of life of a significant percentage of the population. For instance, in the United Kingdom alone, asthma affects around 2 million people. This is a major socio-economic problem with asthma-related prescription costs accounting for approximately £350 million of the National Health Service's annual budget (Rathborne & Naylor, 1996). In 1990, the cost of treating asthma sufferers in the United States of America resulted in a $3.64 billion bill (Weiss, 1992).

1.2 THE DEVELOPMENT OF ALLERGIC ASTHMA

With an increasing awareness of factors that can induce an asthmatic state, clinicians have found it useful to classify asthma according to different aetiological factors. The majority of asthma patients are atopic, i.e., associated with allergy (Custovic, Simpson, Chapman *et al.*, 1998), however the term 'intrinsic' asthma is used for those patients for whom an underlying cause cannot be shown.

In atopic asthma, primary exposure to allergens causes sensitisation. Inhaled, injected or otherwise ingested allergen is presented to the immune system where it is recognised as being foreign, and in susceptible individuals, causes IgE to be produced. IgE is the primary antibody involved in the initiation of immediate allergic responses. The immune system is thus primed so that subsequent exposure to the particular allergen will cause an allergic reaction. When the allergen is again internalised during the secondary exposure, it encounters immune cells, which possess allergen-specific IgE antibodies on their surface. When several IgE molecules bind the allergen, inflammatory mediators are released and the characteristic features of allergic disease follow (Holgate, Church & Lichtenstein, 2000).

1.3 THE SIGNIFICANCE OF ALLERGENS IN THE DOMESTIC ENVIRONMENT

In many countries, allergens derived from dust mites are by far the most important cause of sensitisation, and much of the epidemiological and immunological data available relate to mites (Platts-Mills, Vervloet, Thomas *et al.*, 1997; Squillace, Sporik, Rakes *et al.*, 1997). The process of sensitisation to allergens of the house dust mite (HDM) is directly related to exposure (Kuehr, Frischer, Meinert *et al.*, 1994) and has been found to be the greatest risk factor for developing asthma among middle school children in the USA (Woodcock & Custovic, 1998). Despite the inaccuracy of exposure measurements and the number and variety of allergens and trigger factors (such as cold air, viruses, physical exercise etc.,), it has been possible to make a clear dose-response relationship between

exposure to dust mite allergens and sensitisation (see for example, Sporik, Holgate, PlattsMills *et al.*, 1990; Peat, Tovey, Toelle *et al.*, 1996). It is essential to achieve and maintain a major reduction in allergen levels in the domestic environment to ameliorate symptoms and reduce the risk of sensitisation, although clinical benefits may not be apparent for many months (Custovic, Simpson, Chapman *et al.*, 1998). However, attempts over the last four decades to eradicate HDMs from the typical domestic environment have been largely unsuccessful.

1.4 THE HOUSE DUST MITE (HDM) AND ITS DISTRIBUTION

Traditionally, the common name 'house dust mite' (HDM) has been used collectively to include those members of the family Pyroglyphidae (order Astigmata) that live permanently in house dust. Common names have also been assigned to distinguish between species: European HDM for *Dermatophagoides pteronyssinus* and American HDM for *Dermatophagoides farinae.*

Mites are common in the domestic environment; the most studied and important of these, because of their allergens, cosmopolitan occurrence and abundance in homes are *D. pteronyssinus and D. farinae*. The recommended nomenclature for the allergens of these mites uses the first three letters of the genus (e.g., Der) proceeded by the first letter of the species (e.g. p or f), followed by a number. Allergens from different mite species, but with the same number belong to the same group of allergens and share similarities. These two mite species, together with another important mite species *Euroglyphus maynei*, constitute 90-100% of the total mite population found in mattresses, and 70-95% of the mite population in carpets and soft furnishings (Arlian, 1989). These mites all subsist on dead skin cells and other organic debris in house dust, however discussion in this investigation shall be restricted to the *Dermatophagoides* mites.

The microhabitats of dust mites in the domestic environment are found within textiles, including clothing and soft toys, and upholstered furniture. The weave or pile of fabrics causes dust particles to accumulate and the structure and composition of dust

microhabitats can become complex. House dust consists predominantly of shed human skin scales and fibrous material from fabrics, as well as lesser amounts of various mineral particles derived from soil and building materials (Colloff, 1998). Carpets tend to contain more heavy, large particulate material than dust from upholstered furniture and mattresses, which is predominantly skin scales and fabric fibre.

Dust mites are found in bedding, carpets and upholstered furniture. The main criteria that govern whether mites can live in a particular microhabitat are temperature and humidity and the availability of food. It has become generally regarded that beds contain the most mites and allergens, but this is not the rule (see Colloff, 1998). Due to many factors, including the difference in composition of dust, the topography of the habitat and the area chosen to be representative of the area under study, estimates of dust mite population are difficult to compare between studies. Most survey studies attempt to standardise their sampling procedure when visiting different homes. This usually involves sampling the same region of a habitat with a defined area and sampling for the same amount of time. However there is often a lack of descriptive information to allow proper comparison (Hill, 1998). The method most widely used to determine the abundance of mites in a particular environment is the concentration of Der p1 allergen found which correlates well with the presence of total mite allergens (Chapman, Heymann & Wilkins, 1987) and also Der p2 in dust samples (Sakaguchi, Inouye , Yasueda *et al.*, 1989). This method can also serve as a marker of total mite exposure, although this relation is not simple because the faecal allergen pool is stable and represents past populations of mite as well as current (de Boer, van der Hoeven & Stapel, 1995).

The life cycles of *D. farinae* and *D. pteronyssinus* consist of five life stages: egg, larva, protonymph, tritonymph and adult. Development from egg to adult requires 23 to 30 days under optimum conditions (22-26°C, 75% relative humidity RH) and during their active reproductive life, females produce from 200 to 300 eggs (Wharton, 1976). The time required to complete the life cycle is lengthened or shortened with lower and higher temperatures, respectively. At relative humidities below the optimum, the mite gradually

dehydrates although active life stages are able to survive at ambient humidities as low as 60%RH (Arlian, 1992).

The most important limiting factor for HDM populations is air humidity (Hart, 1998). HDMs osmoregulate through the cuticle and therefore require a high ambient air humidity to prevent excessive water loss. All studies show that the density of mites in homes in humid temperate geographical areas fluctuates sharply with seasonal variations of indoor and outdoor RH (Arlian, 1989). Available epidemiological data on the occurrence of HDMs in the domestic environment demonstrates a clear association between increased indoor air humidity and the increased occurrence of HDMs in house dust. Indoor air humidities below 45%RH for extended periods eradicate HDMs from dwellings (Korsgaard, 1998a).

1.5 MAJOR ALLERGENS OF THE HDM

The major allergens from *D. pteronyssinus* (Der p1 and Der p2) and *D. farinae* (Der f1) that are investigated in this thesis are described below, followed by the major cat allergen, Fel d1.

1.5.1 GROUP 1 ALLERGENS

Two major groups of mite allergen have been defined: the 24 kDa Group 1 allergens and the 14 kDa Group 2 allergens. These groups are structurally unrelated although allergens within each group show extensive amino acid sequence homology and cross-reactivity to antibodies (Heymann, Chapman & Platts-Mills, 1986; Lombardero, Heymann, Platts-Mills *et al.*, 1990). The concentration of Der p1 and Der f1 in the domestic environment ranges from 100 – 10000ngg^{-1} of dust (Platts-Mills & Chapman, 1987; Wahn, Lau, Bergmann *et al.*, 1997), and the minimum amount of Der p1 needed to cause sensitisation and elicitation of symptoms is 2μgg^{-1} of dust (Kuehr, Frischer, Meinert *et al.*, 1994). Group 1 allergens are airborne only during disturbance and are carried on particles >10μm diameter, which precipitate rapidly due to their large size.

Der p1 is the main allergen of the house dust mite *Dermatophagoides pterynissinus*. It is a digestive enzyme that is secreted into the mite's alimentary canal (Thompson & Carswell, 1988). This is the reason why 95% of all dust mite allergen is associated with the faecal particles (Tovey, Chapman & Platts-Mills, 1981). It is also speculated that there is an active secretion of gut contents during feeding, which is supported by the immunostaining of Der p1 in the oesophagus of mites (Tovey & Baldo, 1990; de Lucca, Spovik, O'Meara *et* al., 1999). This would result in the presence of Der p1 in non-faecal material.

The structure of Der p1 is composed of a single chain cysteine protease (Stewart, Thompson & Simpson, 1989), the activity of which is thought to be related to its immunogenicity. In other words, Der p1 is an allergen partly because of its functionality as an enzyme that cleaves other proteins. It has been shown *in vitro* to cause a number of effects observed in allergy and also to increase the permeability of the bronchial epithelium to macromolecules (Herbert, King & Ring, 1995). This increased permeability enables the entry of Der p1, and also those of other allergens. When Der p1 encounters membranes such as the tracheobronchial epithelium, it causes impaired adhesion between the epithelial cells (Robinson, Kalsheker, Srinvasan *et al.*, 1997). This alone would cause a large immune response but the effects of Der p1 do not end there. Beneath the epithelium, Der p1 encounters antigen-presenting cells that interact with T-lymphocytes and trigger a specific immune response. Der p1 also cleaves the low affinity IgE receptor, known as CD23, from the surface of B-lymphocytes. This enhances the IgE immune response by both disrupting the negative feedback regulation of IgE synthesis and by the fact that, once in solution, CD23 promotes IgE production (Hewitt, Brown, Hart *et al.*, 1995). The enhanced synthesis of IgE is the hallmark of an allergic reaction.

Therefore, once Der p1 is inhaled, it invades the body by directly degrading the protective epithelium and initiates an immune response. This immune response is promoted by the action of soluble CD23 and also by the removal of inhibitory

mechanisms. A number of other allergen effects have also been reported due to their protease nature (King, Brennan & Thompson, 1998).

Der f1 also has a single polypeptide chain and shares 81% amino acid homology to, and significant cross-reactivity with, Der p1 (Miyamoto, Oshiwa, Mizimo *et al.*, 1969; Smith, Disney, Williams *et al.*, 1969; Bilotti, Passaleva, Romagani *et al.*, 1972). This partial cross-reactivity suggests the presence of both cross-reacting and species-specific epitopes on Der p1 and Der f1 (Platts-Mills, Heymann, Chapman *et al.*, 1986). Group 1 allergens are heat labile and pH sensitive (Lombardero, Heymann, Platts-Mills *et al.*, 1990) and upon denaturation Der p1 loses its antigenicity (Baldo, Ford & Tovey, 1989).

1.5.2 GROUP 2 ALLERGENS

Der p2 and Der f2 form the second group of major mite allergens. In contrast to the Group 1 allergens, Group 2 proteins are heat stable and pH resistant. (de Blay, Heymann, Chapman *et al.*, 1991). Whereas the antigenic determinants on the Group 1 allergens are readily denatured by heat and appear to be highly dependent on their conformation, Group 2 allergens are resistant to denaturation and only lose their activity after reduction or alkylation (Lombardero, Heymann, Platts-Mills *et al.*, 1990).

Unlike Der p1, Der p2 protein has its IgE-binding activity enhanced by denaturation (Tovey, Ford & Baldo, 1989), which could have been explained by the primary structure of the epitope (Cain, Elderfield, Green *et al.*, 1998). However, by using murine monoclonal antibodies (mAbs), and sera from mite allergic subjects, it has been shown that the epitopes of the Group 2 mite allergens are also conformational and that the three disulphide bonds within the protein stabilise this structure (Smith & Chapman, 1996; Lombardero, Heymann, Platts-Mills *et al.*, 1990). All investigators interested in reducing the concentration of allergens in the domestic environment by denaturation should consider this enhanced IgE-binding activity.

Group 2 allergens are proteins consisting of 129 amino acid residues with a molecular mass of 14kDa and no glycosylation sites (Chua, Doyle, Simpson *et al.*, 1990; Trudinger, Chua & Thomas, 1991). The tertiary structure of Der p2 and Der f2 has shown a protein consisting entirely of β-sheets (Mueller, Smith, Williams *et al.*, 1997; Ichikawa, Hatanaka, Yuuki *et al.*, 1998), and these proteins share 88% amino acid sequence homology (Yuuki, Okumara, Ando *et al.*, 1991; Chua, Doyle, Simpson *et al.*, 1990). Both murine and human antibodies recognise common epitopes on both allergens and have been reported to have almost complete cross-reactivity (Yasueda, Mia, Yui *et al.*, 1989).

Group 2 allergens are reported to be present at an approximately five-fold lower concentration than Group 1 allergens in mite extracts and spent mite medium, but are found in relatively higher amounts in mite bodies (Ford, Rawle, Lind *et al.*, 1985). At present, the functions of Group 2 allergens in the mite are not known. Der f2 could possibly be a component of the innate antibacterial defence system of mites because it binds to the surface of the bacterium *Escherichia coli* (Ichikawa, Hatanaka, Yuuki *et al.*, 1998). Airborne Group 1 and Group 2 mite allergens behave aerodynamically similarly to each other. Group 2 allergens, possibly carried on fragments of mite cuticle, are airborne only during disturbance and precipitate rapidly.

1.6 THE MAJOR CAT ALLERGEN, FEL D1

One in four homes in the United Kingdom contain a cat, *Felus domesticus* (Custovic, Simpson, Pahdi *et al.*, 1998), and it is suggested that as many as one third of cat-sensitised individuals live in homes with a cat. Up to 40% of children with asthma are sensitised to cat allergen (Gelber, Seltzer, Bouzoukis *et al.*, 1993) and even brief exposure to cats can precipitate severe asthma symptoms in susceptible individuals. It has been reported that airborne Fel d1, the major cat allergen, can be detected in all houses with cats and in almost a third of homes without cats (Custovic, Simpson, Pahdi *et al.*, 1998). High levels have also been reported in schools (Enberg, Shamie, McCullough *et al.*, 1993).

Fel d1 is a 36kDa protein, which is produced primarily in the sebaceous glands and in the basal squamous epithelial cells of the skin. It is mainly stored on the surface of the epidermis and fur (Charpin, Mata, Charpin *et al.*, 1991) and a single cat can produce between 3-7μg per day (Dabrowski, van der Brempt, Soler *et al.*, 1990). Similar to the Group 2 mite allergens, Fel d1 (and also the major dog allergen, Can f1) is heat stable (Cain, Elderfield, Green *et al.*, 1998).

Unlike Group 1 mite allergens, Fel d1 can remain airborne in undisturbed conditions and is associated with a large range of particle sizes, including particles <2.5μm in diameter (Luczynska, Li, Chapman *et al.*, 1989). In some houses, it has been reported that the levels of Fel d1, associated with small particles, were comparable to the quantities previously reported to produce acute airway obstruction in provocation experiments, i.e., 8-80ng of Fel d1 inhaled during 2 minutes (Ohman, Findlay & Leitermann, 1984; van Metre, Marsh, Adkinson *et al.*, 1989). It is likely that these properties are responsible for the distinct rapid onset of symptoms experienced by patients who are allergic to cats (de Blay, Chapman & Platts-Mills, 1991).

1.7 METHODS OF ALLERGEN AVOIDANCE

It is important to remove, or at least prevent, exposure to allergens from the domestic environment in order to prevent sensitisation or the elicitation of symptoms. At very low allergen levels, conversion from sensitisation to desensitisation may also occur (Wickman & Korsgaard, 1996). The primary method of reducing exposure to cat allergen is the removal of the cat from the living room and bedroom areas of the home. The use of High Efficiency Particulate Air (HEPA) filters can reduce airborne levels of cat allergen in homes with cats. However, the reduction following cat removal has been found to be solely due to the precipitation of the larger particle sizes (Custovic, Simpson, Pahdi *et al.*, 1998). Airborne cat allergen appears to be maintained by resuspension of particles from reservoirs within the room such as carpets and soft furnishings (de Blay, Chapman & Platts-Mills, 1991). Repeated washing of cats not only removes allergen but also leads to

progressive reductions in the quantity of allergen accumulating on the cat (Glinert, Wilson & Wedner, 1990).

Beds, flooring, clothing and soft toys can harbour considerable colonies of mites (Tovey, Chapman, Wells *et al.*, 1981), and Der p1 is very stable in domestic environments (Kort & Kniest, 1994; de Boer, van der Hoeven & Stapel, 1995). Mechanical disturbance, especially household cleaning, can result in a significant rise in airborne particles from these dust reservoirs (Clark, 1974). Encasing mattresses and pillows with occlusion covers gives the best protection from the bed's large mite population and the methods recommended for cleaning clothes, bedding and soft toys include: frequent hot washing, dry cleaning, prolonged freezing, heat sterilisation and storage under dry conditions (Tovey, 1992).

Steam cleaning of carpets has been recommended for effective reduction of both mites and allergen concentrations (Colloff, Taylor & Merrett, 1995). The use of liquid nitrogen, combined with intensive vacuum cleaning, is highly effective at killing and removing mites in mattresses and carpets decreasing their population by 90-100% (Colloff, 1986). Freezing with liquid nitrogen however has no effect on allergens. This procedure requires specialist application and needs to be repeated. Even though carpets can be temporarily rendered mite free, they cannot be sufficiently cleared of all dust, are easily re-infested, and can serve as a collection and distribution point for mite aeroallergen from other sources. There is therefore no substitute for frequently washed hard floors if minimal allergen exposure is to be achieved.

Chemicals can be used to control mite populations and include acaracides such as benzyl benzoate. While these compounds can kill mites *in vitro*, there is difficulty in applying them effectively to the resident mite population in the matrix of mattresses, carpets and soft furnishings. There is also a possibility that adverse skin reactions to the chemicals may occur whilst either in close physical contact, enclosed spaces, or with repeated applications for extended periods (Tovey, 1992).

The use of tannic acid to denature allergens is very effective at treating surface dust (Green, Nicholas, Salome *et al.*, 1989). Although when combined with an acaricide and applied to beds, floors and furnishings, the denatured surface allergens are replaced by dust and mites from deeper in the matrix (Tovey, Marks, Matthews *et al.*, 1992). Vacuum cleaners are useful to extract excess surface dust from items and remove it from the circulating pool of aeroallergen. Although some studies have shown reductions of mites and dust by repeated vacuuming (Burr, Dean, Merrett, 1980), such treatment is not an effective method for controlling either dust or mites in carpets, soft furnishings or mattresses. However, a recent study has shown that applying an electrostatically charged carpet powder before vacuuming significantly increases the amount of dust removed from the carpet dust reservoir (Jerrim, Hughes & McKechnie, 2001).

Increasing ventilation and venting sources of moisture to the outside are simple ways of controlling the level of humidity in the domestic environment (Niven, Fletcher, Pickering *et al.*, 1999). Some studies have shown clinical benefits of using HEPA filters (Colloff, Ayres, Carswell *et al.*, 1992) even though no clinical improvement was found when electrostatic air precipitators were used to reduce the aeroallergen concentration (Mitchell & Elliot, 1980). Although HEPA filters were more effective than electrostatic ones, medical specialists recommend neither in the absence of other forms of environmental control (Nelson, Hirsch, Ohman *et al.*, 1988).

The use of ionisers in the treatment and prevention of asthma will be discussed in *Section 1.11*. Ionisers utilise corona discharge to produce ions. This process is discussed in detail in the following section.

1.8 CORONA DISCHARGE

1.8.1 DEFINITION

Corona discharge occurs when a high voltage is applied between electrodes in a non-uniform geometry (Cross, 1987) e.g. when one electrode has a small radius of curvature, a point, and the other is a plane (see *Figure 1.1*). By definition a corona is a discharge where the ionisation is non-thermal and confined by geometry to narrow ionisation region(s) close to the point electrode(s) (Sigmond & Goldman, 1978).

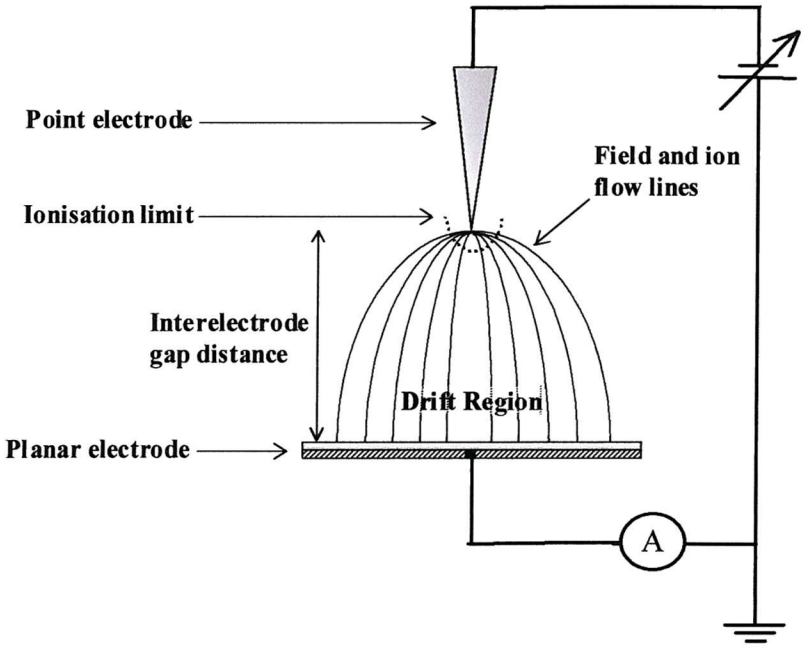


***Figure 1.1* Illustration of a point-to-plane corona discharge.**

The non-uniform electric field causes an electrical discharge in the high-field region near the point, at voltages below the spark breakdown of the inter-electrode gap. In this region, the electric field is very high and exceeds the breakdown field of the gas. Any electron entering the region becomes sufficiently accelerated, so that in the event of a collision with an atom it can detach another electron. This electron is itself accelerated,

producing its own electron/ ion pair near the point. During this ionisation process, light is emitted and, in air, a bluish glow is visible around the sharp electrode, thus giving the discharge its name 'corona'.

1.8.2 NEGATIVE CORONA DISCHARGE

The behaviour of the discharge depends on the polarity of the point electrode because of the stark difference in nature of positive ions and electrons. When a negative high voltage is applied to a point electrode in air, naturally occurring negative ions that enter the high-field region split to form an electron and a neutral atom. The electron accelerates away from the negative point, due to the high electric field, and gains sufficient energy to ionise any atom with which it collides. This electron multiplication process continues until the new electrons are formed at a distance so far away from the high-field region that they are unable to gain sufficient energy from the electric field between collisions to ionise any atoms. At the same time, the positive ions that are produced are attracted to the negative point electrode, gaining energy from the field. When these collide with the electrode they release secondary electrons, which add to the discharge.

In gases containing electronegative atoms, such as oxygen or fluorine, electrons that have insufficient energy to form an electron/ positive ion pair attach to a neutral atom to form a negative ion. In negative coronas in air, O_3^-, CO_3^- and O^- are the most commonly produced ions (Taylor & Secker, 1994), although $O_3^-(H_2O)$, $CO_3^-(H_2O)$, $O_2^-(H_2O)_2$, $O_2^-(H_2O)_3$, CO_4^-, $CO_4^-(H_2O)$, NO_2^-, $NO_2^-(H_2O)$ and NO_3^- are also produced by reactions in the interelectrode gap (Bastien, Haug & Lecuiller, 1975). These ions are the main charge carriers, which contribute to current flow outside the ionisation region. In non-electronegative gases, such as pure nitrogen, electrons form the current in a negative corona and the current is much higher than that observed in air (Cross, 1987).

The drift of ions to the anode, under the influence of the electric field, results in collisions with neutral gas molecules and imparts momentum to them, creating the *ion wind*. These neutral molecules are induced into motion in the direction of the electric field, giving rise

to bulk movement of the air. This wind is created by energy transfer from the ions and is not necessarily the movement of the ions themselves. The ion wind transmits up to 50% of the discharge power to the planar electrode, in the form of heat and chemically very potent excited atoms and molecules. These atomic and molecular radicals are known as *neutral metastable species* and are produced by free electrons in the electrode space. These electrons disappear, by dissociative attachment, forming the chemically important neutrals such as those shown in the equations below (Lecuiller, Julien & Pucheault, 1972; Goldman, Goldman & Sigmond, 1985):

$$e^- + O_2 \rightarrow O^- + O$$

$$e^- + CO_2 \rightarrow O^- + CO$$

$$O^- + H^+ \rightarrow OH$$

$$O_3^- + H^+ \rightarrow O_2^+ + OH$$

$$CO_3^- + H^+ \rightarrow CO_2 + OH$$

Other active species that are produced by negative corona in an aqueous solution are H, HO_2 and H_2O_2 (Sun, Sato & Clements, 1997). The metastable species have very short half-lives, e.g., O is very reactive and has a half-life of only 100μs (Demuth, 1984). Research into the development of radicals in an aqueous solution by the arrival at the surface of negative ions, has found that the ion species is dependent on the regime of the discharge such as Trichel, or negative continuous glow (Lecuiller, Julien & Pucheault, 1972). The hydroxyl radical (OH) is known to play an important role in killing bacteria and degrading organic compounds (Sun, Sato & Clements, 1997). Its production increases exponentially with applied voltage and is produced in greater quantities in positive coronas (Ono & Oda, in press). Another product of both negative and positive coronas (3-5% of the total corona products) is ozone (Demuth, 1984).

1.8.3 POSITIVE CORONA DISCHARGE

When the point electrode is positive, electrons created by ionisation are attracted to the point and positive ions drift towards the plane. The positive ions undergo many collisions with neutral molecules and lose energy but do not cause further ionisation. No secondary

electrons are formed as they drift towards the cathode with a much reduced energy. The electrons created in the ionisation region travel towards the positive point forming avalanches of electron/ ion pairs in the gas. Again, most of the current in the drift region is carried by ions of the same polarity as that of the point (Cross, 1987). The ions most commonly produced in a positive corona under atmospheric and ambient conditions are hydrated, such as $H^+(H_2O)_n$, $NO^+(H_2O)_n$, and $NO_2^+(H_2O)_n$ (Taylor & Secker, 1994); less frequently $OH^+(H_2O)_n$, $CO_4^+(H_2O)_n$, and $O_2^+(H_2O)_n$ are produced (Pethig, 1983).

A recent study has shown that the ions $H^+(H_2O)_n$, NO^+, O_2^+, $H_2O^+(H_2O)_5$ are found near the planar electrode during positive pulse corona (Held & Peyrous, 1999). A greater variety of ions are found near the planar electrode however during positive continuous glow corona: $H^+(H_2O)_n$, O_2^+, NO^+, CO_2^+, N_2O^+, N_2H^+ and less abundantly: $H^+(H_2O)_n$, N_2^+, NO_2^+. In dry air, only N^+, O^+, N_2^+, and O_2^+ are produced (Held & Peyrous, 1999).

1.8.4 CORONA REGIMES

Corona discharge is a generic term for a spectrum of different types, or regimes, of corona. In negative coronas, even in weakly electronegative gases, the interplay between positive and negative space charges within the electrode space leads to the current pulsations known as *Trichel pulses*. If the corona current is increased, these pulsations are damped down to produce the *continuous glow* corona (Sigmond & Goldman, 1978). In positive coronas, the burst pulses will either join together, becoming a regularly pulsating *positive glow* (*Hermstein glow* or *ultracorona*) covering the active electrode, or the largest will develop into *positive streamers*, which are plasma filaments that carry their own ionisation region ahead and propagate rapidly towards the cathode (Sigmond & Goldman, 1978). Negative streamers are only important in parallel plate gaps and long rod-to-plane gaps. This is because in atmospheric air approximately $1.8MVm^{-1}$ is needed for stable propagation, as opposed to about $0.5MVm^{-1}$ for positive streamers (Sigmond & Goldman, 1978). The visual characteristics of the different positive corona regimes, taken with a digital camera, can be seen in *Figure 1.2*.

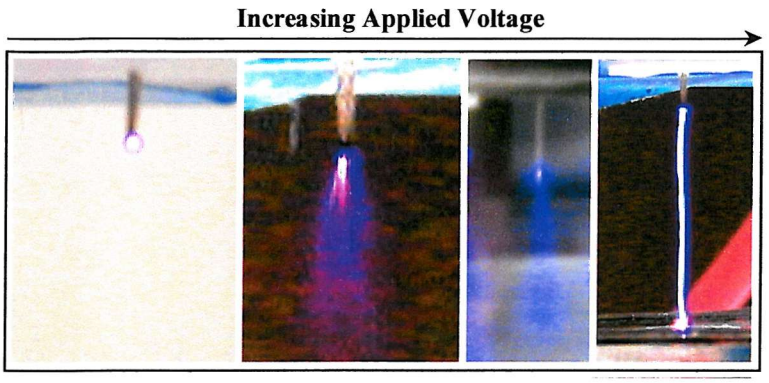


***Figure 1.2* Photographs showing the visual characteristics of each positive corona regime with increasing applied voltage to a point-to-plane electrode geometry.** At the onset of corona and during positive pulse little light is emitted but with increasing voltage (A) the glow can be seen to be localised at the tip of the point electrode. With increasing applied voltage, positive glow stabilisation occurs due to a localised plasma emitting photons around the point electrode, then (B) the plasma can grow into a self-propagating filament containing a weakly conducting plasma channel. These filaments can be stable but if the voltage is increased further they may become onset-streamers and (C) even reach the cathode, exposing the planar surface to high energy bursts of ions. If the voltage is increased even further (D) the breakdown limit of the gas may be reached and a non-corona spark breakdown may occur.

The corona current-applied voltage characteristics are dependent upon interelectrode gap distance, point radius of the electrode and atmospheric RH and pressure. These have been investigated by Miyoshi (Miyoshi, Hosokawa & Sakai, 1964). A number of other variables can affect the nature of corona discharge. It has been suggested that most corona surveys are preferably conducted by one experimenter in order to ensure the constancy of these unknown parameters while varying the known (Sigmond & Goldman, 1978). Although techniques have greatly improved, including the ability to analyse corona products more effectively and use computer simulations to determine chemical reactions, the general consensus of authors in the field is that our understanding of the processes of corona discharge is still in a rudimentary state.

1.9 ELECTROCHEMICAL SURFACE REACTIONS IN CORONAS

Corona discharges are widely used as chemical reactors for surface treatment. An industrially very important application is the surface treatment of polymers. This treatment is used to increase the polymer's wettablility and adhesivity to ease printing, painting, sealing, coating etc. (Goldman, Goldman & Sigmond, 1985). The efficiency of a corona surface treatment depends upon the polarity of the corona. Negative coronas are generally more efficient than positive coronas for oxidation and etching (Sigmond, Goldman & Brenna, 1980). Corona products transfer their energy to the polymer by breaking chains and creating radicals. Polar bonds and hydrogen bonds formed in this way will increase the polymer's surface energy. The bonds most frequently encountered are C-O, C=O, C-O-O$^-$, C-OOH (carboxyl group) (Briggs, 1983), and in smaller amounts, NH (amino group), NO, NO_2 and NO_3 (Demuth, 1984). The effect of the corona treatment increases with time and current until a saturation point is reached; then oxidation processes are counterbalanced by decarboxylation processes, as revealed by the ejection of CO, CO_2 and H_2 molecules emitted from the surface of the polymer (Laurent, Mayoux, Noel *et al.*, 1983). The chemical changes in the surface often cause changes in the structure.

As early as 1893, Thomson commented on the similarities between current-conducting gases and liquid electrolytes (Thomson, 1893). In both types of media the current conducting mechanisms are fundamentally interconnected with chemical processes, while electrochemical reactions are induced at the interfaces between the media and the metal electrodes (Goldman, Goldman & Sigmond, 1985).

Negative coronas in air of more than 10-20%RH will rapidly form a semi-liquid layer on anodic metal surfaces. This layer contains water and NO_x^- ions from the air as well as dissolved impurities. Powered by the corona ion current, this layer behaves like any other aqueous electrolyte cell, as illustrated in *Figure 1.3* (Goldman, Goldman & Sigmond, 1985). With aluminium as the anode, pronounced pitting occurs and aluminium reaction products are formed. When these products were dissolved in hot water, the ions Al^{3+} and NO_3^- were found (Sigmond, Goldman & Brenna, 1980). Under dry conditions, negative

air coronas cause only uniform anodic passivation of this metal, building up surface layers charged to several hundred volts.

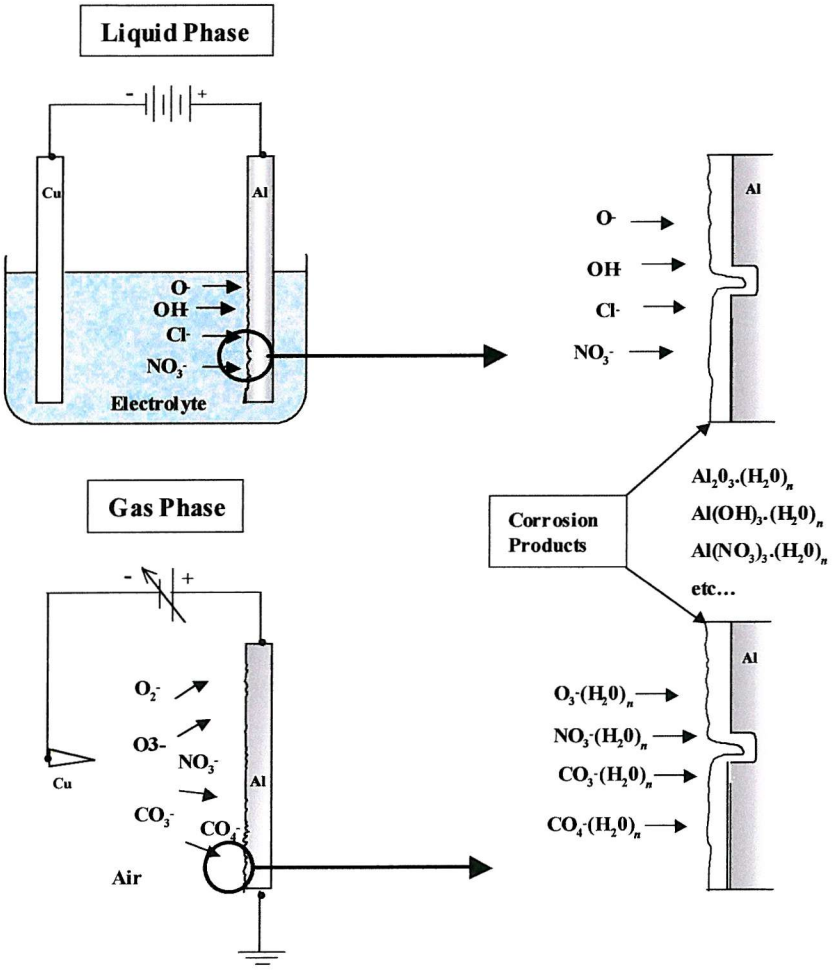


***Figure 1.3* Illustration of the similarities between negative corona discharge and electrolysis.**

The pitting activity of negative ions lead to comparable effects on aluminium anodes in an electrolyte and in gaseous corona discharge. (Based on Goldman, Goldman & Sigmond, 1985).

1.10 IONISERS IN THE MANAGEMENT OF ASTHMA

In the late 1950's/ early 1960's Kreuger *et al.*, researched the effect of ionisers on the respiratory system of anaesthetized laboratory animals and isolated tissue. He reported that the influence of positive ions could cause a sensitive organism to develop a condition similar to that of an asthma attack (Kreuger & Smith, 1960; Kreuger, 1962). Negative ions, on the other hand, were seen to reverse the harmful effects caused by the positive. Further investigations led to the discovery that O^- and CO_2^+ are the mediators of any physiological effects that occur in the trachea, as a result of atmospheric ionisation (Kreuger & Smith, 1960).

Research into treating asthma sufferers with a therapy consisting of exposure to negative ions led to varying success (Field, Schulte, Mikat *et al.*, 1961; Kornbleuh & Griffin, 1955; Kornbleu, Piersol & Speicher, 1958). Although some researchers reported positive results in one half of their attempts to treat asthma attacks with negative ions, the effect, if any, was thought to be very small and possibly due to the psychological effects of the treatment. Attempting to eliminate the psychological factor from their investigations, experiments were devised in order to determine the effect of positive and negative ionisation of the surrounding local atmosphere on infants below the age of one (Palti, De Nour & Abrahamov, 1966). The results of these tests were optimistic and led to the conclusions that negative ions caused:

- Shorter duration of an asthma attack, as compared with the duration of the attack in children receiving conventional treatment
- Reduction of tachypnoea (increased breathing rate) in cases of bronchospasms
- Termination of attacks induced by positive ionisation.

Positive ions were found to:

- Induce attacks in normal infants
- Cancel the therapeutic effects of negative ions.

Although the previous studies had suggested a beneficial effect of negative ions on lung function in asthmatics, the observed changes were always minor (Jones, O'Conner, Collins *et al.*, 1976). Some researchers also found that a slight improvement in lung function could also be seen with positive ions (Osterballe, Weeke & Albrechtson, 1979). Unfortunately poor regard for control subjects is a major criticism of early experiments into the effect of ions on the respiratory system.

More recently, Ben-Dov *et al.*, studied the response of 17 asthmatic patients, aged between 10-20 years, to exercise while breathing negatively ionised air under controlled conditions (Ben-Dov, Amirav, Shochina *et al.*, 1983). Their results showed that exercise-induced asthma was significantly attenuated in the test in which negatively ionised air was breathed during exercise. This reduction in exercise-induced bronchial reactivity occurred in all but one of the patients tested.

Controlled investigations into the effect of positive ionisation of inspired air on bronchial reactivity have also been performed (Lipin, Gur, Amtai *et al*, 1984). The results showed that positively ionised air aggravated exercise-induced asthma in eight out of the twelve subjects tested. The concentration of positive ions was of the same order as the negative ions in their previous study, i.e., $5 - 10 \times 10^5$ ionscm^{-3} (Ben-Dov, Amirav, Shochina *et al*, 1983). From these studies it was concluded that the ionic charge of the air could possibly influence the bronchial response to exercise and that this, in addition to the other known variables such as temperature and humidity, could be a modifying factor.

However, Nogrady and Furnass (1983) performed a six month, double blind, crossover study into the benefits of using negative ion generators on 20 subjects with stable asthma. After an initial two week period without an ioniser, active or placebo ionisers were installed in the subjects' bedrooms for two eight week periods, separated by a four week period when no ioniser was present. From the results of their study, these investigators concluded that there were no significant differences in the subjects' peak expiratory flow rate (PEFR), symptom score or consumption of medication throughout the experiment. Thus, negative ion generators imparted no significant benefit to asthmatic subjects.

Nogrady and Furnass suggest that due to the ability of negative ion generators to enhance the precipitation of airborne particulates including allergenic dust, any benefit shown in earlier studies could have been attributable to this phenomenon. However, one study using electrostatic precipitators has failed to show any significant benefit for asthma sufferers (Mitchell & Elliot, 1980). This study has also been criticised because any improvement gained might have been offset by the production of the positive air ions that the precipitator used (Podleski, 1980). This idea is also contentious and some think that the biological effect of air ions is so small, even in concentrations far exceeding natural levels, that it is difficult to measure their effects. Additional difficulties include controlling and measuring all the environmental factors that may influence the experiment (Ball, 1980). Nogrady and Furnass also suggested a possible reason why their study did not show any significant improvement in the health of the subjects – the production of ozone by the corona discharge-utilising ionisers. Ozone is known to have a bronchoconstrictor action and would thus mask any beneficial actions of the negative ions. Although they mentioned that during bench testing of the ionisers they could not detect ozone from two metres away, no values of ozone production, or how it was measured, were given.

Thus, the use of ionisers alone is not at present recommended for the use in the home for the symptomatic relief of asthma (Collof, Ayres, Carswell *et al.*, 1992).

1.11 THE DELETERIOUS EFFECT OF OZONE ON HEALTH

Ozone is an air pollutant that has been recognised as an important public health hazard. People believed to be at particular risk for the adverse effects of ozone include those with chronic pulmonary disease and asthma (Paulo & Gong, 1991; Lippman, 1989). Epidemiological studies have also demonstrated reductions in the pulmonary function of both asthmatics and non-asthmatics with increased levels of ambient ozone (Bates & Sizto, 1987; Lioy, Vollmuth & Lippman, 1985). Thus ozone may play a significant role in the exacerbation of airway disease in asthmatics. Long-term exposure may also cause

irreversible lung destruction and cancer (Reiser, Tyler, Hennessy *et al.*, 1987; Witschi, 1988).

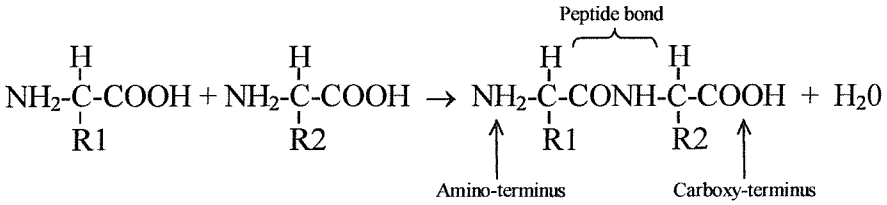
Studies into the effect of ambient ozone concentrations on non-allergic and non-asthmatic volunteers have shown that exposure to ozone results in a decrease of pulmonary function and induces a neutrophilic influx into the lower airways (Koren, Devlin, Graham *et al.*, 1989; Devlin, McDonnell, Mann *et al*, 1991; Folinsbee, Mc Donald & Horstman, 1988). Although, research has not yet been able to fully define the mechanism(s) by which ozone exposure may act to alter asthmatic airway inflammation or bronchospasm, present research does suggest that ozone can induce airway inflammation in humans.

Another study has shown that ozone, like allergen after a clean air exposure, acts as a stimulus for eosinophil influx into nasal secretions (Bascom, Naclerio, Fitzgerald *et al.*, 1990). These researchers performed allergen challenges in patients with allergic rhinitis after exposure to clean air or 0.5ppm ozone for 4 hours. The results of their nasal lavages also showed that ozone has an intrinsic inflammatory action that mimics, to a certain degree, the effect that allergen challenge can have in subjects with allergic rhinitis.

1.12 PROTEIN STRUCTURE

Most allergens are proteins (including those studied in this investigation). Proteins are polymers formed by condensation reactions between amino acids. Once incorporated into a protein the amino acids are termed residues. There are twenty amino acids used to make proteins and all have individual structures because they all have different functional groups (R). The R group is called the side chain of the amino acid.

The bond between amino acid residues is termed the peptide bond and is formed in the condensation reaction illustrated below:



The function of a protein is determined by, and is dependent on, its three-dimensional structure. The shape of a protein is determined first by its primary structure, i.e., the sequence of amino acid residues. The symmetrical folding of the polypeptide chain determines the secondary structure, e.g., the α-helix, or the β-sheet, and the tertiary structure is the way the various secondary structures fold about each other. (Stryer, 1995).

A protein can become denatured by the presence of a number of agents that can disrupt the three-dimensional structure (see *Figure 1.4*). Changes in pH, or salt concentration, alter the electrostatic interactions between charged amino acids and also interfere with hydrogen bonding between residues. Increasing the temperature reduces the strength of hydrogen bonds, and the presence of reducing agents leads to the breaking of disulphide bonds between cysteine residues within the molecule. The primary structure of a protein remains intact when it has been denatured because none of these agents break the peptide bonds between amino acid residues.

When a protein has been denatured, it loses its function. Often, when a protein has been gently denatured and is then returned to its optimum conditions of temperature, pH, salt concentration etc., it can spontaneously regain its function, or become renatured. This happens due to spontaneous resumption of its native three-dimensional shape, which is intrinsic to the protein, i.e., no outside agent is required to refold it. This is due to the fact that the final shape of the protein is due to the precise sequence of amino acid residues and their electrostatic interactions within the molecule (Wada and Nakamura, 1981).

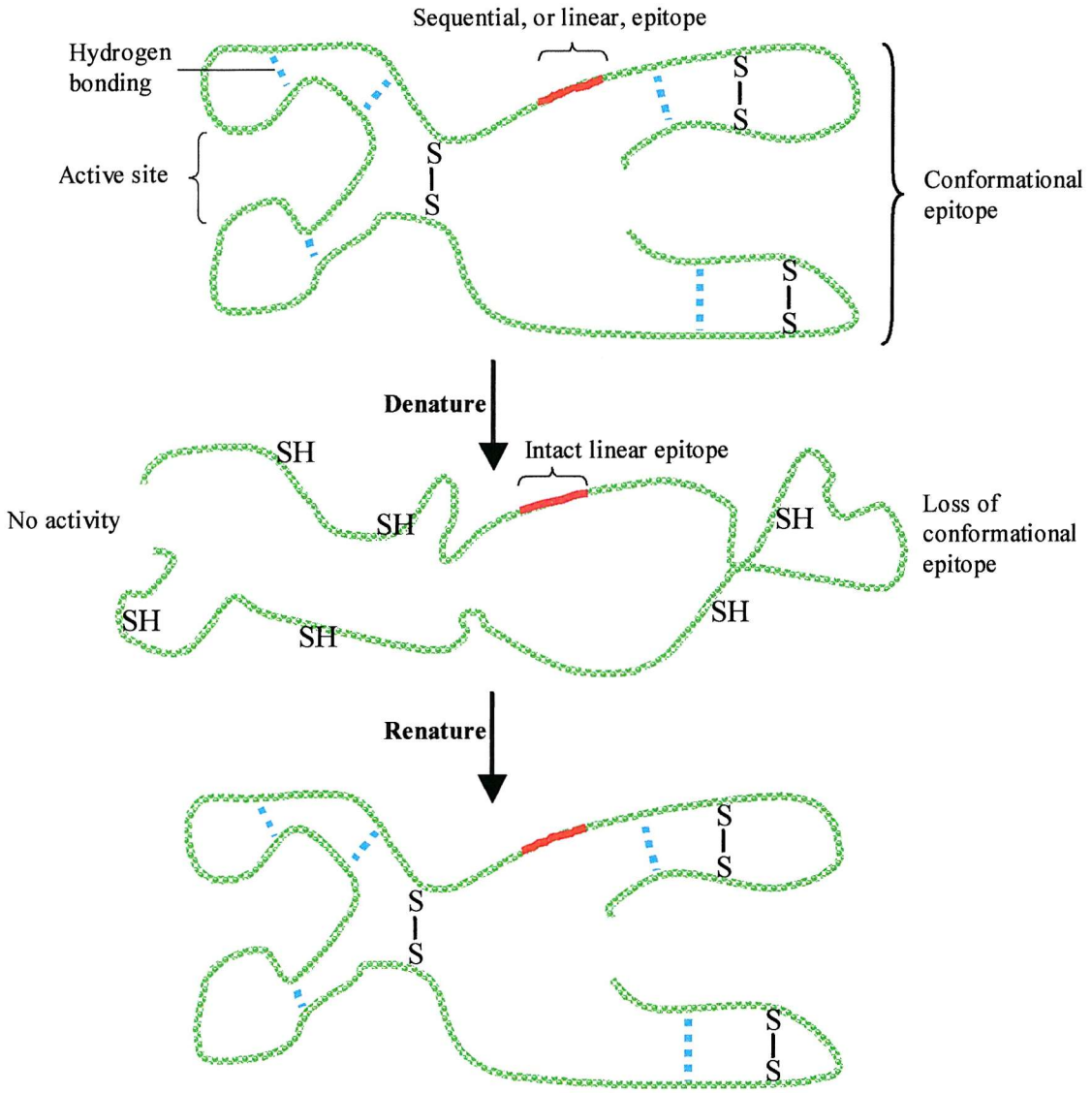


Figure 1.4. **Diagram illustrating the denaturing and renaturing of a protein.**

The normal structure of the protein, stabilised by hydrogen bonding and disulphide bonds becomes denatured by, for example, increased temperature or lower pH, thus reducing the disulphide bonds (S-S) and weakening the hydrogen bonding. In this state, any conformational epitopes, or active sites, will be lost. However linear epitopes, consisting of a sequence of amino acid residues contiguous to each other, will not be altered. Upon return to optimum conditions, the protein will renature and return to its native form, regaining all activity.

Together with hydrogen bonds and electrostatic interactions, disulphide bridges may form between cysteine residues. The amino acid cysteine, shown in *Figure 1.5*, is important in the allergens studied in this investigation because of the three disulphide bonds used to stabilise the conformational epitopes of Group 1 & 2 allergens (Smith & Chapman, 1996; Lombardero, Heymann, Platts-Mills *et al.*, 1990) and the cysteine residue at the centre of the active site of Group 1 allergens (Chua, Stewart, Thomas *et al.*, 1988).

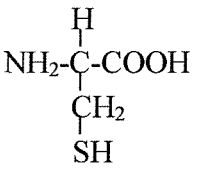


Figure 1.5. The amino acid cysteine.

1.13 AIMS OF THE PRESENT INVESTIGATION

As the link between HDM occurrence and atopic asthma is now well established (Korsgaard, 1998b), methods of reducing exposure to HDM allergen is important in the avoidance and control of atopic disease. Ionisers have been investigated for any potential clinical benefits they may have, however, the potential allergen-destroying efficacy of corona discharge has not previously been investigated.

The aims of this investigation were to determine whether corona discharge had a detrimental effect on the integrity of the major household allergens Der p1, Der f1, Der p2 and Fel d1 and whether it could translate well into a practical application for reducing allergen concentration in the domestic environment. Alterations to the structure of the allergen would result in the protein's loss of activity and the failure of the allergen to be recognised by the immune system, thereby reducing the risk of sensitisation and/ or the elicitation of the symptoms of allergic disease.

The method most commonly used to investigate the effects of a treatment on household allergens is to use a heterogeneous powder mixture containing the desired allergen. This is usually spent dust mite culture medium, or sieved house dust collected from vacuum cleaner bags. Preliminary experiments into the effect of corona discharge on sieved dust mite culture, although encouraging, didn't allow detailed studies (*Section 2.3*). The aim of the investigation into the variability of Der p1 concentration in samples aimed to improve the concentration homogeneity of samples and allow detailed investigation of the effect of corona discharge on allergens (*Section 2.4*). The resultant technique was then used in the following investigations.

Using a corona electrode apparatus of constant point radius, interelectrode gap and surrounded by a constant ambient temperature, investigations to determine the effects of corona discharge were performed. Studies into the effect of initial allergen concentration were performed in order to determine whether the amount of allergen present on the planar electrode affected the percentage reduction and number of molecules of allergen destroyed per unit exposure (*Section 3.2*).

Experiments into the effect of the duration of exposure to corona discharge, using different corona regimes and polarities (*Section 3.3*), the magnitude of the corona current (*Section 3.4*), and the relative humidity of the surrounding atmosphere (*Section 3.5*) were performed in order to characterise the response of the different allergens to these variables of corona discharge. The powerful oxidizing corona product, molecular ozone, was also investigated using an ultra-violet (U.V.) lamp to produce the gas in order to determine whether this was responsible for the effects of corona discharge on the allergens (*Section 3.6*). Ozone is deleterious to health; therefore if it were not involved in the mechanism of corona-destruction of allergens then future attempts to reduce this would be beneficial. Concentration analysis of the allergen under test was performed using Enzyme-Linked Immunosorbent assay (ELISA) throughout the investigations.

Aluminium foil was used as the planar electrode in all tests. However, this does not remain inert during corona irradiation but reacts with the corona products to produce a

number of aluminium oxides (see *Section 1.9*). In order to determine the effect that these products may have upon the allergen, and also on the integrity of the biological components of ELISA, experiments were designed aimed at testing these potential artefacts (*Section 3.7*). Der p1 was tested to see whether, after exposure to corona discharge, the allergen could renature (*Section 3.8*). This would give an indication of the mechanism of corona-destruction of this allergen, i.e., if the protein's structure is altered due to loss of hydrogen bonding within the structure or whether more permanent alterations are made due to chemical modification or cleavage of the protein's primary structure.

Due to the encouraging results of the previous investigations, the final stage in the series of experiments aimed at determining whether the technique of corona-destruction could translate well into a practical application for the removal of allergens from the domestic environment. Chamber tests of an experimental nine-pin ion wind generator, or a small commercially available ioniser (designed for use in wardrobes to remove odours) were performed in order to determine whether Der p1 samples could be destroyed at larger distances away from the corona source over different time periods (*Section 4.3*).

This experimental ion wind generator and a larger commercially available ioniser (designed for cleaning the air of large rooms) were also tested in room-scale exposures using an unoccupied, furnished office room. This enabled the determination of the efficacy of the ionisers under conditions more closely resembling an *in situ* application (*Section 4.8*). This latter test utilised distances of up to 4m away from the corona source.

In *Section 4.9*, an investigation is described that aimed at determining the extent to which any corona products, responsible for the allergen-destroying effects observed in previous experiments, could penetrate into different fabrics and foams used in soft furnishings. This is important to investigate because the main allergen reservoir in the domestic environment is present inside mattresses and soft furnishings. A successful application of corona discharge to remove allergens from the home would have to include this property of penetration.

CHAPTER 2: PRELIMINARY EXPERIMENTS AND INVESTIGATION OF DER P1 CONCENTRATION VARIABILITY IN SAMPLES

MATERIALS AND METHODS

2.1 DETERMINATION OF THE CURRENT-VOLTAGE CHARACTERISTICS OF THE PIN-TO-PLANE CORONA SET-UP

As described in *Section 1.8*, the behaviour of corona discharge depends upon variables such as RH and the applied voltage. In a pin-to-plane corona set-up, just after corona onset, the ions will drift from the point to the planar electrode producing pulses in the corona current. Depending on the polarity of the applied voltage, these pulses are either called negative Trichel corona, or positive pulse corona. As the applied voltage is increased the corona current also increases and the pulses will become more frequent and dampen down until negative, or positive, continuous glow corona is produced. The magnitude of the corona current will be greater in continuous glow regimes due to the greater variety of ions produced (Held & Peyrous, 1999). If the applied voltage is increased further, high-energy streamers will be produced provided the electrode geometry and conditions are appropriate.

In order to investigate any effect that these different regimes might have upon the allergen content of a sample, it was necessary to determine the corona current-applied voltage characteristics of the electrode arrangement used. By plotting the current-voltage characteristics, it was possible to determine the onset of each regime together with the range of corona currents and applied voltages at which they occur in the experimental conditions used.

2.1.1 CONSTRUCTION OF THE POINT-TO-PLANE CORONA ELECTRODES

A simple point-to-plane corona apparatus was made using Perspex 10mm wide (see *Figure 2.1* for a photograph and schematic diagram). A frame was made with the Perspex and a stainless steel pin (point radius 45μm) was inserted through the centre of the top. The lower surface of the frame was then covered with a sheet of aluminium to form the planar electrode. This produced a fixed interelectrode gap of 15mm. A frame of aluminium, coated on the upper surface with insulating tape, with a $20mm^2$ square removed from the centre was then placed over the planar electrode and hinged at one side. This frame was used to locate samples of allergen solution, which had been evaporated onto aluminium foil firmly to the planar electrode (see *Chapter 3*). The planar electrode could then be connected to earth and direct current (d.c.) high voltage of the desired polarity applied to the pin using a high voltage generator (Model 3807: Alpha Series III, *Brandenberg Ltd.*, Surrey, England).

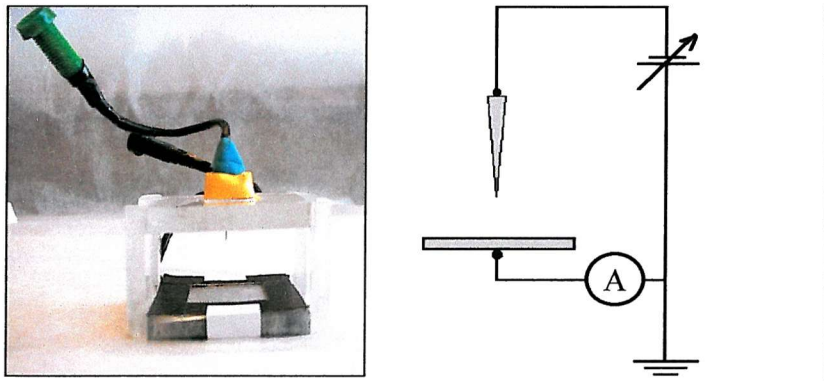


***Figure 2.1* Photograph and schematic diagram of the point-to-plane corona electrode apparatus used to investigate the effects of corona discharge on allergens.**

2.1.2 MEASUREMENT OF THE CURRENT-VOLTAGE CHARACTERISTICS OF THE POINT-TO-PLANE CORONA ELECTRODES

The corona apparatus was placed on a 12mm thick Teflon base and the point electrode was connected to a d.c., high voltage supply. The voltage was recorded with an electrostatic voltmeter (SCALAMP, *WG PYE & Co Ltd.*, Cambridge, England). The planar electrode was connected to earth through a microammeter (Universal Avometer, Model 8 Mk 7, *MEGGER INSTRUMENTS Ltd*, Dover, England).

A $30mm^2$ piece of aluminium foil was fixed to the planar electrode and the high voltage generator was switched on. The applied voltage was increased in 0.5kV increments every five seconds until the breakdown voltage of the interelectrode gap was reached. At each increment the corona current was recorded. This was repeated three times at constant RH values of 2.4%, 10%, 20%, 45% and 50%. After each test, a digital oscilloscope (Model: TDS 210, *TEKTRONIX INC.*, Beaverton, Or., USA) was connected in the ground line to verify the onset of the pulse or continuous glow regime and to record the visual characteristics of each regime.

To achieve relative humidities of 20, 45 and 50% the experiment was performed in a temperature and humidity-controlled room using a combined dehumidifier (Model: MD08, *Munters*, Huntingdon, England) and humidifier (Defensor, Model: 2000-V, *Munters*, Huntingdon, England). To perform the experiment at 10%RH, the corona electrodes were sealed inside a glass vessel with compressed air entering the vessel at a low velocity of $0.25ms^{-1}$ to ensure that the pressure was not raised above atmospheric pressure (10^5Pa) inside the vessel (see *Figure 2.2* for a photograph of the apparatus). The RH of the compressed air was constant at 10%. To repeat the experiment at 2.4%RH, the compressed air was first dried by being passed through a 1m glass tube filled with colour-indicating silica gel. The results are shown in *Section 2.5*.

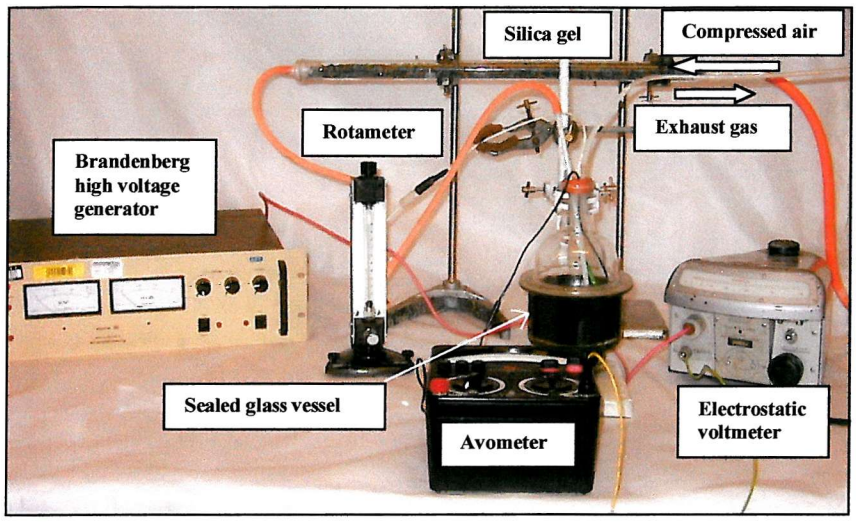


Figure 2.2 **Photograph of the apparatus used to determine the current-voltage characteristics of the point-to-plane corona electrodes in an atmosphere of 10% and 2.4%RH.**
Compressed air was passed through the silica gel and into the sealed glass vessel containing the corona electrodes at a fixed rate. The Brandenberg high voltage generator was then turned on and the applied voltage to the point electrode measured using the electrostatic voltmeter. The corona current from the ground line was measured using the Avometer. No silica gel was necessary to achieve an RH of 10%.

2.2 PREPARATION OF HOUSE DUST MITE (HDM) CULTURES

Cultures of HDMs were obtained by placing material obtained from British domestic vacuum cleaner bags that tested positive for Der p1 by ELISA (Enzyme-Linked Immunosorbent Assay) using anti Der p1 monoclonal antibody (mAb) 5H8 and biotinylated anti Group 1 mAb 4C1 (INDOOR Biotechnologies Ltd., Cardiff, UK) (Luczynska, Arruda, Platts-Mills *et al.*, 1989) into 0.5 litre jars containing culture medium. Cultures require a container, an area-enlarging substrate, high protein food and favourable temperature and humidity (Wharton, 1976). These conditions were met by using 465g of finely ground dog biscuit as the food and 14g (3% of the total mass) of dried yeast as the substrate. The humidity was maintained in equilibrium at 75%, and the temperature was kept at a constant 25°C (optimum HDM conditions) by using a tray of saturated saline solution ($NaCl_{(aq)}$) placed at the bottom of the incubator. The size of the cultured mite population was assessed by removing a small sample for viewing under the microscope to count mites and by ELISA to obtain a value of Der p1 concentration. Once the culture had become established for several months it was frozen to kill any living mites and so arrest further allergen production.

2.3 EXPOSURE OF SIEVED, SPENT HOUSE DUST MITE CULTURE TO NEGATIVE CONTINUOUS GLOW CORONA DISCHARGE USING A PIN-TO-PLANE ELECTRODE ARRANGEMENT

The point-to-plane corona electrodes (described in *Section 2.1.1*) were used in this test. Onto the planar electrode, 400μg of finely sieved (<63μm) culture medium was placed using a spatula and half of this was then removed, weighed and kept as the control in ambient air for the duration of the experiment. The remaining sample was then redistributed evenly over the planar electrode. The pin was connected to a negative d.c. high voltage supply (Model 3807: Alpha Series III, *Brandenberg Ltd.*, Surrey, England) to create a corona discharge with a corona current of 90μA. Samples were exposed for 60, 120, 180, 240 and 300 minutes. The sample was then carefully removed, weighed and diluted 100-fold with 1% Bovine Serum Albumin in 0.05% Phosphate-Buffered Saline with Tween20 (BSA-PBS-T) in preparation for ELISA. The control dust, previously removed, was treated the same. This protocol was repeated to produce 12 replicates. Any solid particulates that accumulated on the pin during corona discharge were brushed into an eppendorf and added to 200μl 1% BSA-PBS-T and prepared for ELISA. The relative humidity was constant at 45% and the temperature was constant at 25°C. Results are shown in *Section 2.6.*

2.4 INVESTIGATION OF DER P1 SAMPLE VARIABILITY

This investigation was performed to determine the extent of Der p1 concentration heterogeneity. Methods of allergen distribution and preparation are investigated to determine the most consistent method of preparing Der p1 samples for exposure to corona discharge.

2.4.1 PREPARATION OF AN AQUEOUS SOLUTION OF HDM CULTURE

A HDM culture was prepared according to *Section 2.2* and sieved to below 63μm. 15g of this culture fraction were then added to 400ml of distilled water. This mixture was stirred well to enable all the Der p1 to dissolve, then passed through filter paper (*Whatman*, qualitative, grade 4) to remove solid material. Thimerosal (sodium ethylmercurithiosalicylate) was added at a concentration of 0.001% to prevent bacterial, fungal and mould growth.

2.4.2 DISTRIBUTION OF CULTURE MEDIUM PARTICLES WITH A SPATULA OR SIEVE.

A circular disc of aluminium foil (70mm diameter) was divided equally into 8 sectors. 1g of sieved (<63μm) culture medium was then spread as evenly as possible over the foil using a spatula. The culture medium in the individual sectors was then brushed into an eppendorf tube and 1% BSA-PBS-T was added to produce a 1 in 100 dilution for subsequent testing for Der p1 concentration by a two-site monoclonal antibody ELISA This procedure was repeated twice. Results are shown in *Section 2.7*, *Figure 2.8*.

In order to assess the importance of the dust distribution technique, 1g of sieved culture medium was evenly distributed over the foil discs using a fine sieve (63μm pore size), and then removed as above and prepared for ELISA. This was also repeated twice. Results are shown in *Section 2.7, Figure 2.9.*

2.4.3 DISTRIBUTION OF THE AQUEOUS SOLUTION OF CULTURE MEDIUM

Another application method employed an aqueous solution of the culture medium prepared according to *Section 2.4.1.* 300ml of this solution were then poured onto an aluminium foil-covered 300mm x 230mm tray and dried at 37°C. Two circular pieces of foil of 70mm diameter were then cut out of the foil at random positions. Each were then cut into 8 sectors and placed in an eppendorf. 1% BSA-PBS-T was added to give a 1 in 200 dilution in preparation for testing by ELISA. Results are shown in *Section 2.7, Figure 2.10.*

The final Der p1 application method to be investigated also used the culture medium solution, prepared as above. Sixteen 100µl aliquots of this solution were micropipetted onto foil and left to evaporate in a drying oven at 37°C. These were diluted 6-fold with 1% BSA-PBS-T and prepared for ELISA. Results are shown in *Section 2.7, Figure 2.11.*

In order to determine whether evaporation of the aqueous solution of Der p1 had any detrimental effect on the concentration itself, twelve 100µl samples were evaporated at 37°C and diluted in 1% BSA-PBS-T as above and then assayed by ELISA. These samples were then compared to the Der p1 concentration of twelve 100µl drops that were placed directly into an eppendorf and diluted 6-fold in 1% BSA-PBS-T without being evaporated. Results are shown in *Section 2.7.*

RESULTS AND DISCUSSION

2.5 THE CURRENT-VOLTAGE CHARACTERISTICS OF THE POINT-TO-PLANE CORONA ELECTRODES

Figures 2.3 & 2.4 show the applied current-voltage characteristics of the point-to-plane corona electrodes during negative, or positive corona. These graphs clearly show the dependence of the I/V response upon the atmospheric RH. As the RH was increased the I/V response curve was shifted to the right and the maximum corona current was decreased. The inflexion points that indicate the transition from negative Trichel or positive pulse to continuous glow corona generally occurred at higher applied voltages for higher values of RH.

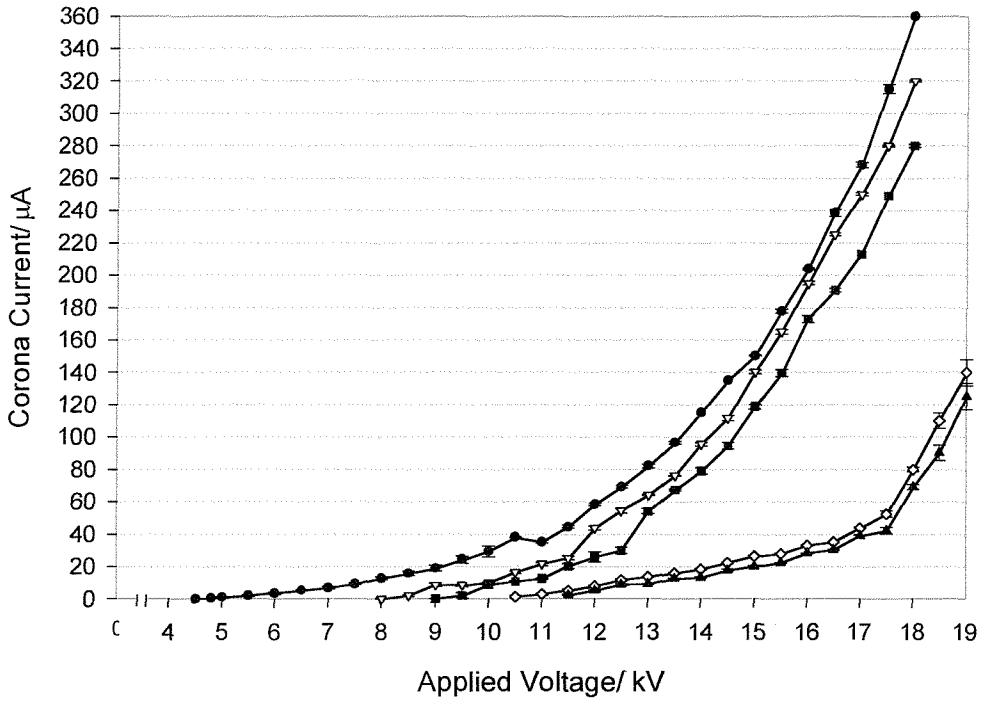


***Figure 2.3.* The applied voltage-corona current characteristics for negative point-to-plane corona at atmospheric pressure and at different relative humidities.**
● = 2.4%RH, ▽= 10%RH, ■ = 20%RH, ◇ = 45%RH, ▲= 50%RH. The interelectrode space was 15mm with a point radius of 45µm (n = 3, SEM shown).

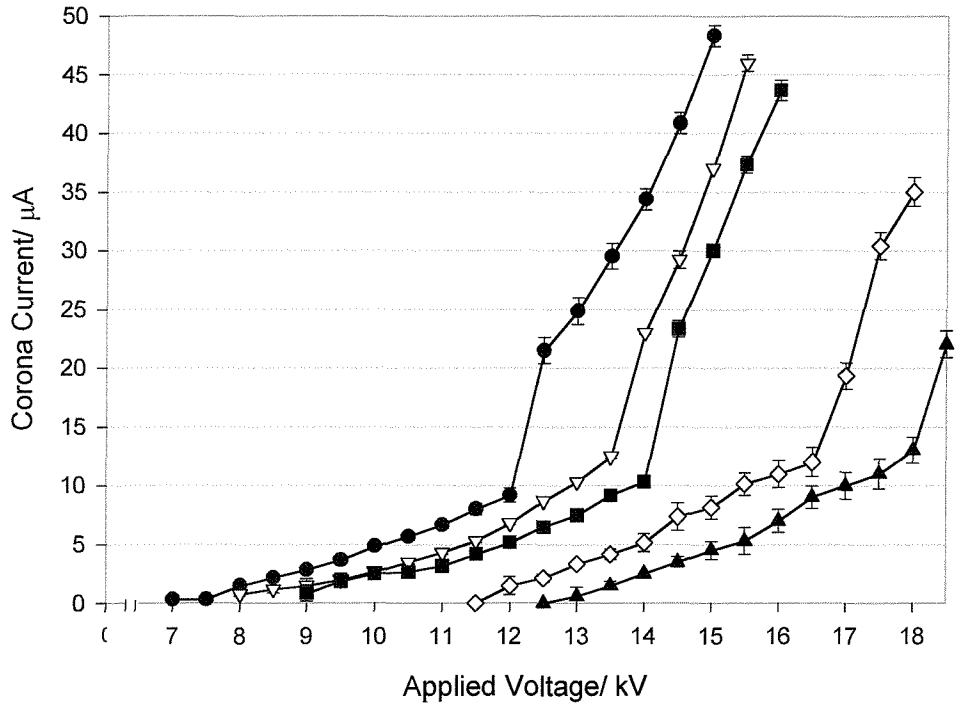


Figure 2.4 **The applied voltage-corona current characteristics for positive point-to-plane corona at atmospheric pressure and at different relative humidities.**
● = 2.4%RH, ▽= 10%RH, ■ = 20%RH, ◇ = 45%RH, ▲= 50%RH. The interelectrode space was 15mm with a point radius of 45μm (n = 3, SEM shown).

The corona currents and applied voltages of the onset of continuous glow corona are shown in *Table 2.1*. An oscilloscope trace of negative Trichel corona is shown in *Figure 2.5*. The transition from negative Trichel, or positive pulse to continuous glow corona indicates the onset of production of a greater quantity and variety of ions (see *Section 1.8*). This resulted in the greater increase in corona current observed for each applied voltage increment compared with the Trichel and positive pulse regimes.

RH	Negative		Positive	
	I/ μA	V/ kV	I/ μA	V/ kV
2.4	35.00	11.0	21.5	12.5
10	43.87	12.0	23.00	14
20	54.00	13.0	23.33	14.5
45	80.00	18.0	19.32	17
50	69.12	18.0	33.00	18.5

Table 2.1 **The corona currents and applied voltages at which continuous glow corona begins.**
Values are the mean of 3 replicates.

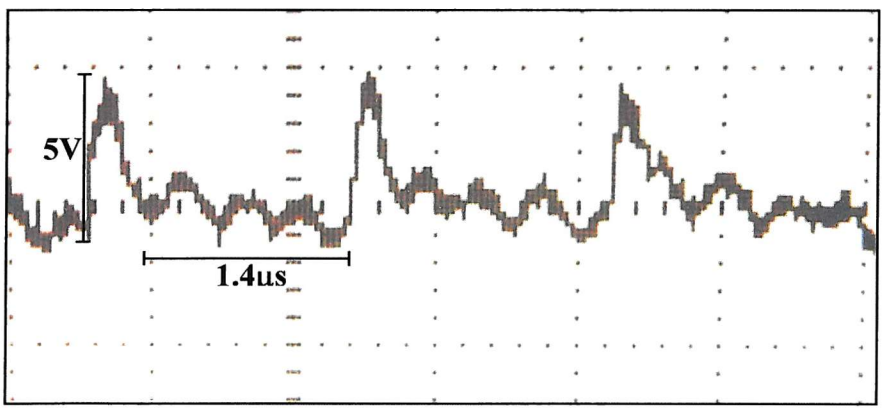


Figure 2.5 **An oscilloscope trace of typical Trichel pulses.**

Due to the differences in the current-voltage characteristics at different RH values, a current was chosen, at each RH, to be representative of the corona regime for later investigation. For the negative Trichel corona regime, a corona current of 15μA was chosen for the investigation of the effect of RH on the allergen-reducing efficacy of corona (see *Sections 3.5 & 3.13*) and 25μA was chosen at an RH of 45% for all other

experiments performed in this investigation. For negative continuous glow, a current of 90μA was chosen. Corona currents of 5μA and 30μA were chosen to represent the positive pulse and positive glow regimes respectively under RH conditions of 2.4, 10, 20 and 45%. At an RH level of 50%, a corona current of 20μA was chosen to represent positive glow.

2.6 EXPOSURE OF DER P1-CARRYING FAECAL PARTICLES TO CORONA DISCHARGE

Figure 2.6 shows the Der p1 concentration in the samples exposed to negative continuous glow corona with a corona current of 90μA for different exposure times and their paired controls. When the concentration data was analysed (see *Appendix* for a description of the statistical tests) using the t-test paired for means, or the Mann-Whitney-U test where appropriate, only the samples exposed for 180 minutes were statistically lower than their controls (P=0.045). This was probably due to the large variation in allergen content of the controls.

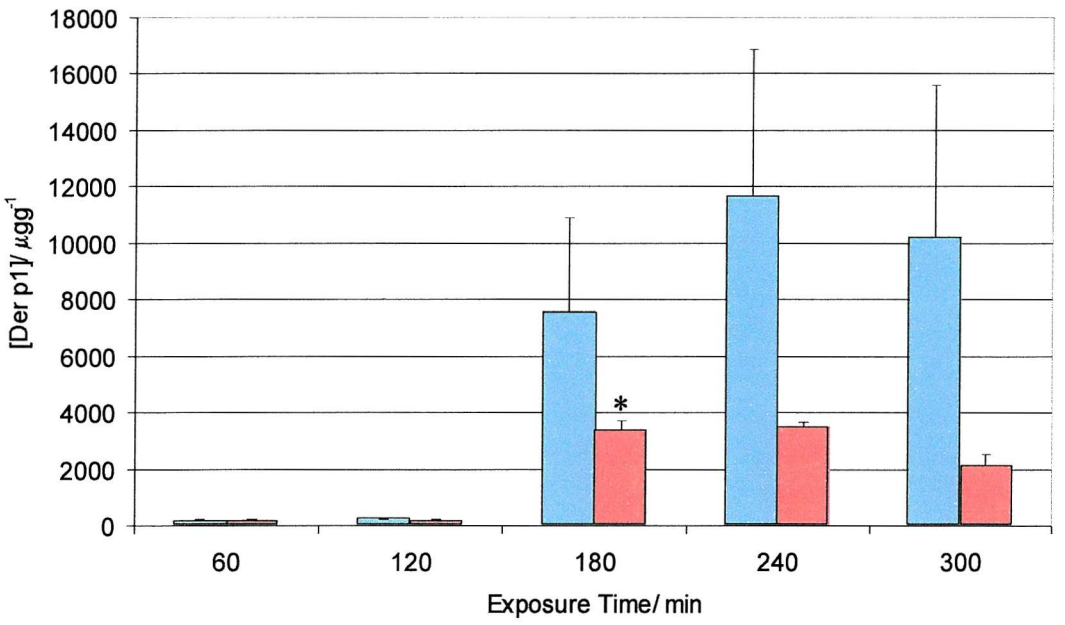


***Figure 2.6* The Der p1 concentrations in the samples exposed to negative discharge and their controls**
Samples (■) were exposed to 90μA, negative continuous glow corona for varying exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period. These experiments were performed on heterogeneous, sieved HDM culture (*= statistically lower than the control, n=12, SEM shown).

Figure 2.7 shows the relationship between the mean percentage reduction in Der p1 concentration and the length of exposure to corona discharge. The percentage reductions increased from −1.90 ±8.99% after 60 minutes exposure through 4.11 ±7.07%, 19.83 ±9.45%, 21.22 ±12.46% and 24.17 ±13.62% after 120, 180, 240 and 300 minutes exposure respectively.

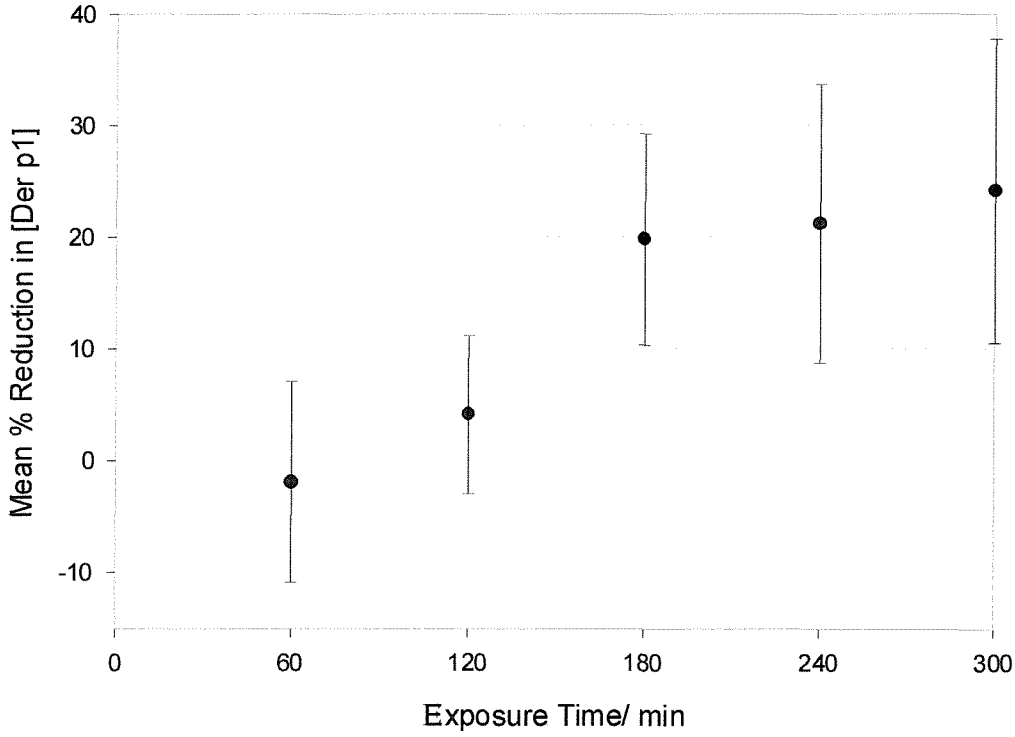


***Figure 2.7* The percentage reduction in Der p1 concentration of the sieved, HDM culture after exposure to negative continuous glow corona.** (n=12, SEM shown).

The cause of the variability was probably due, in part, to the use of a heterogeneous sieved culture as the source of the allergen. Heterogeneous powder mixtures inevitably lead to concentration variability and the exact concentration of the samples tested could not be predicted before the corona exposure. This lack of concentration homogeneity can

lead to difficulties in distinguishing the effects of any compound or technique on the concentration of the allergen in the sample and even lead to false results. Spurious effects are well known using this method, such as an increase in concentration of the allergen in a sample that has been treated with a technique known to reduce the allergen (see for example, Colloff, Ayres, Carswell *et al.*, 1992).

Another problem encountered during corona discharge on the dry culture medium was the loss of sample to the surrounding environment. Electrostatic charging and field effects led to a visible accumulation of solid material on the upper electrode. The total amount was small, weighing less than 0.1mg. However by adding this to 200µl of 1% BSA-PBS-T, the ELISA determined that the sample consisted of 23.92ng of Der p1.

The particles of the powder mixture on the planar electrode became electrostatically charged as a consequence of exposure to uni-polar ions. Particle movement within the interelectrode gap was subsequently induced by the electric field. This explains the behaviour of the powder escaping the corona set-up or being attracted to the negative polarity of the upper electrode. The allergen could also have been attracted to the negative polarity of the upper electrode because of the Der p1-carrying faecal particles' inherent net positive polarity under dry conditions (Gaynor, Hughes, 1998).

Due to these aforementioned problems, the 12 replicates were insufficient to obtain a statistically significant value for the amount of Der p1 destroyed under corona irradiation for the majority of time exposures. The following sections investigate different methods of allergen sample preparation for the analysis of any effects that corona discharge may have upon the allergen.

2.7 INVESTIGATION OF DER P1 SAMPLE VARIABILITY

The Der p1 concentration can be seen to vary considerably in all tests using sieved culture medium. *Figure 2.8* shows the Der p1 concentration of sectors with allergen sample distributed with a spatula. The Der p1 concentration of most sectors varied from 32.45μgg^{-1} to 99.63μgg^{-1} but sectors 7 and 8 had a much greater concentration of 604.81μgg^{-1} and 618.81μgg^{-1} respectively.

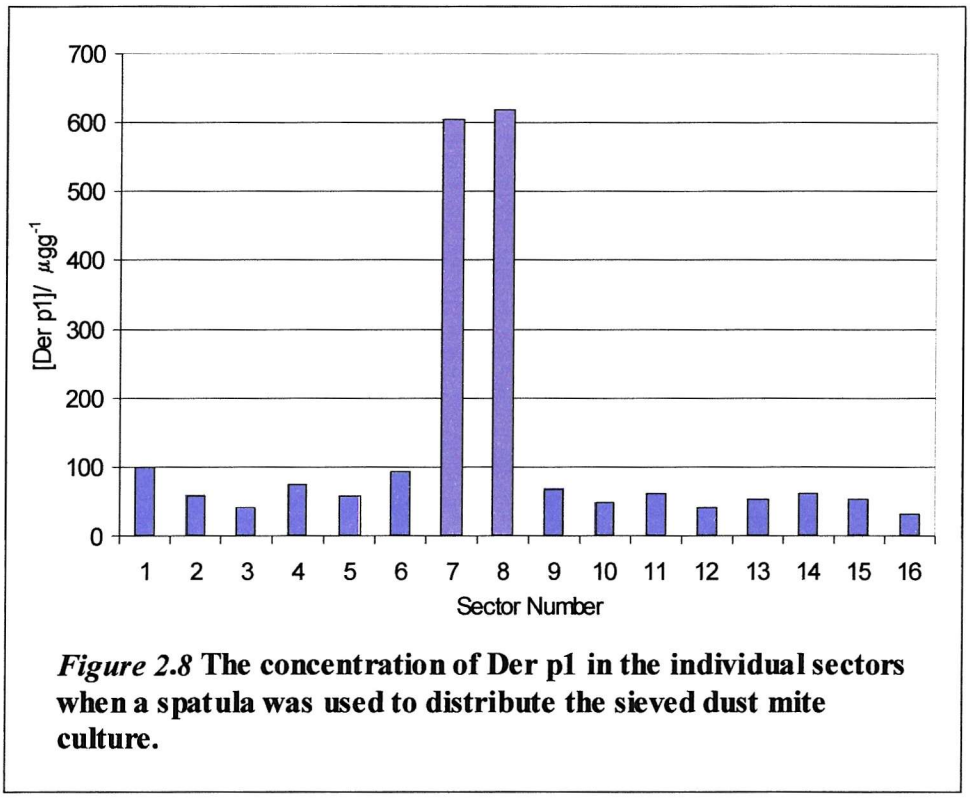


***Figure 2.8* The concentration of Der p1 in the individual sectors when a spatula was used to distribute the sieved dust mite culture.**

Using a sieve to spread the allergen samples (see *Figure 2.9*) led to Der p1 concentrations that varied from 5.91μgg^{-1} to 83.27μgg^{-1}. Allowing 300ml of the Der p1 solution to evaporate on foil led to a greater variability in concentration than using a sieve to distribute solid particles (see *Figure 2.10*). The Der p1 concentration varied from 6.04μgg^{-1} to 64.80μgg^{-1}.

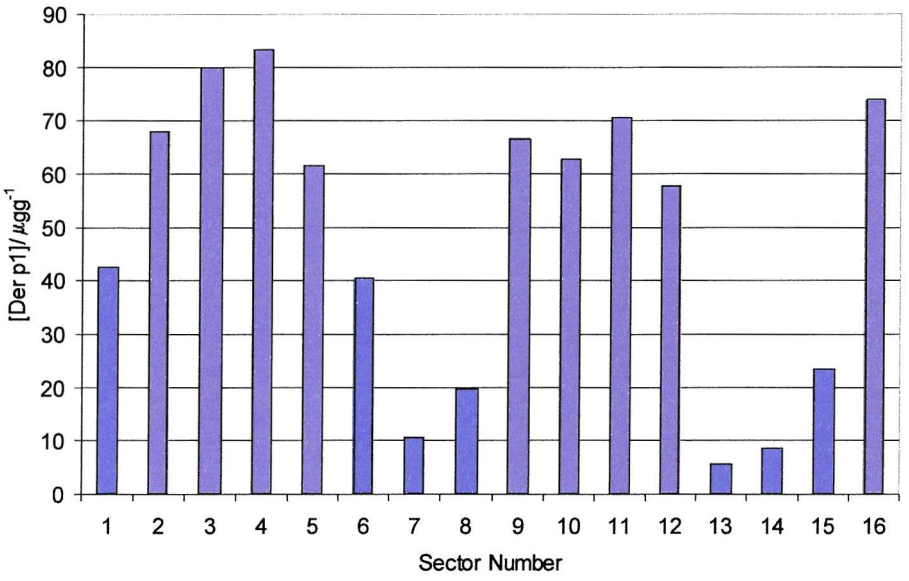


Figure 2.9 **The concentration of Der p1 in the individual sectors after a <63 µm pore sieve was used to distribute the sieved dust mite culture.**

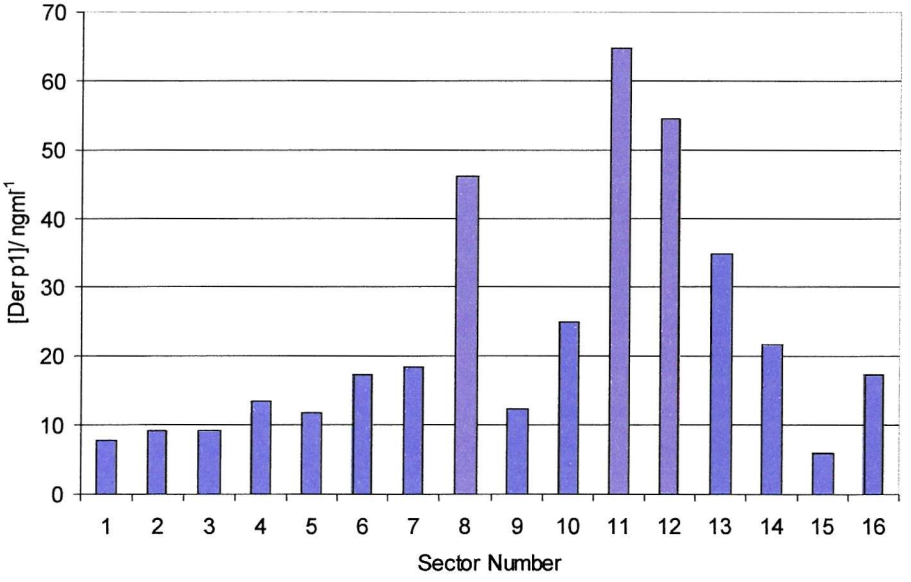


Figure 2.10 **The concentration of Der p1 in the individual sectors after 300ml of Der p1 solution was evaporated onto a tray.**

However, using the evaporated 100µl drops of Der p1 solution led to a very uniform concentration throughout the samples tested (see *Figure 2.11*). The concentration of samples varied from 54.43ngml^{-1} to 77.03ngml^{-1}; a range of only 22.60ngml^{-1}, which is a more acceptable variability and corresponds to the uniform nature of homogeneous solutions.

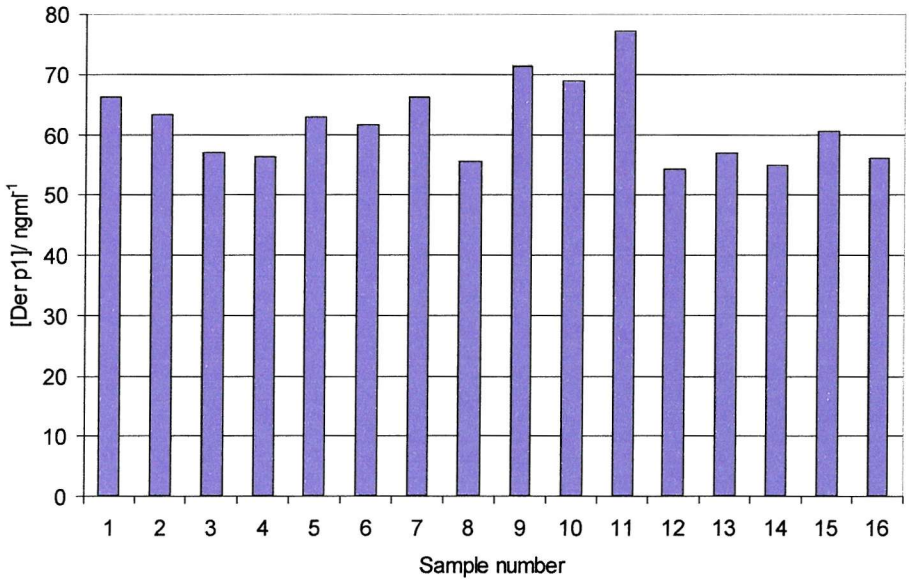


***Figure 2.11* The concentration of Der p1 in the individual 100µl aliquots.**

Table 2.2 shows the coefficient of variation between sectors for each of the methods of sample application. These values show that the concentration variability of a Der p1 sample distributed by a spatula is the greatest followed by the evaporating 300mls of Der p1 solution onto a tray method; using a sieve and the aliquot method varied least. The 100µl aliquot method gave a concentration variation more than 10-fold less than using a spatula to distribute the sample. The results from the experiment to determine whether evaporation reduced the concentration of Der p1 showed no difference between the two sets of samples.

Method of sample application	Spatula	Sieve	Tray	Aliquot
CV	146.07	55.55	77.04	10.82

***Table 2.2* The Coefficient of Variation of the samples' concentration taken from the experiments using different methods of Der p1 distribution.**

Using an aqueous solution of dust mite culture also has the advantage over using sieved house dust because of the possible contamination of the dust with accidental or purposely added chemicals. Studies have indicated that chemical treatment of carpets to reduce dust mite allergen as well as the presence of household detergents commonly recovered from house dust can affect the performance of ELISAs (Dybendal, Vik & Elsayed, 1990; Chew, Higgins, Milton *et al.*, 1999).

As a result of these tests, and due to the problems encountered in *Section 2.6*, the evaporated aliquot method was adopted as the preferred method to investigate the effect of corona discharge on a number of different allergens presented in the following sections. This method has the advantage that the samples may be firmly attached to the planar electrode of a corona apparatus and so the allergen sample would not be lost to the atmosphere, nor migrate to the upper electrode as observed in *Section 2.6*. Another advantage is that different electrode configurations may be tested such as wire-to-cylinder and wire-to-plane, as the samples may be placed vertically or be inverted. Vertically positioned samples are used in *Sections 4.3 & 4.4*.

CHAPTER 3: SHORT-RANGE EXPOSURE OF EVAPORATED ALLERGEN SOLUTION SAMPLES TO CORONA DISCHARGE USING A PIN-TO-PLANE ELECTRODE ARRANGEMENT

MATERIALS AND METHODS

3.1 PREPARATION OF AQUEOUS SOLUTIONS OF DER P1, DER P2, DER F1 AND FEL D1 ALLERGENS

Differing masses of dust, or HDM culture, were used to produce concentrations of the desired allergen at approximately 100 $ngml^{-1}$. This was calculated from knowledge of the allergen content of the powder tested using the appropriate two-site monoclonal antibody ELISA.

Solutions of *Dermatophagoides pteronyssinus* allergens were made using a HDM culture, prepared according to *Section 2.2* and sieved to below 63μm. 15g of this culture fraction were then added to 1.5litres of distilled water to produce a solution of Der p1. To produce an equivalent concentration of Der p2, 150g of this fraction was added to 1.5litres of distilled water. A solution of the *Dermatophagoides farinae* allergen Der f1 was prepared using material obtained from American domestic vacuum cleaner bags that had tested positive for Der f1 by ELISA. This material was sieved to below 63μm and 2g of this fraction were then added to 1.5litres of distilled water. A solution of the *Felus domesticus* allergen Fel d1 was made using material obtained from British domestic vacuum cleaner bags that had tested positive for this allergen. This material was also sieved to below 63μm and 1.6g of this fraction were added to 1.5litres of distilled water.

All mixtures were stirred well to enable the allergens to dissolve, then passed through filter paper (*Whatman*, grade 4) to remove solid material. Thimerosal (sodium ethylmercurithiosalicylate) was added to each solution at a concentration of 0.001% to prevent bacterial, fungal and mould growth.

These allergen solutions were then assayed by the appropriate two-site monoclonal antibody (mAb) ELISA kits (INDOOR Biotechnologies Ltd., Cardiff, UK): Der p1 concentration was determined using the Der p1 ELISA kit utilising anti-Der p1 mAb 5H8 and biotinylated anti-Group 1 mAb 4C1, (Luczynska, Arruda, Platts-Mills *et al.*, 1989); Der f1 concentration was determined using the Der f1 ELISA kit utilising anti-Der f1 mAb 6A8 and biotinylated anti-Group 1 mAb 4C1, (Luczynska, Arruda, Platts-Mills *et al.*, 1989); Fel d1 concentration was determined using the Fel d1 ELISA kit utilising the anti-Fel d1 mAb 6F9 and biotinylated mAb 3E4 (Chapman, Aalberse, Brown *et al.*, 1988); Der p2 concentration was determined using mite Group 2 ELISA kit utilising the anti-Group 2 mAb 1D8 and biotinylated mAb 7A1 (Ovsyannikova IG, Vailes, Li *et al.*, 1994). As the anti- Group 2 allergen antibody is fully cross-reactive with Der f2, the presence of the latter allergen was also checked.

Twenty 100µl aliquots were removed from each allergen solution and prepared for ELISA by placing them each into an eppendorf and adding 500 µl of 1% BSA-PBS-T to produce a 1 in 6 dilution. Results of this concentration analysis are shown in *Section 3.9.*

3.2 THE EFFECT OF INITIAL ALLERGEN CONCENTRATION UPON THE EFFICACY OF NEGATIVE CORONA DISCHARGE TO DESTROY DER P1, DER F1, DER P2 AND FEL D1

These experiments into the effect of initial allergen concentration were performed in order to determine whether the amount of allergen present on the planar electrode affected the percentage reduction and number of molecules of allergen destroyed per unit exposure to negative corona discharge.

Allergen solutions containing Der p1, Der f1, Der p2 and Fel d1 were prepared according to the protocols outlined in *Section 3.1*. These were then diluted using distilled water to produce concentrations ranging from 40 – 1800ngml^{-1}. The allergen samples were then prepared by pippetting 100µl onto 30mm^2 squares of aluminium foil. The sample and its paired control were prepared adjacently. These were then dried at 37°C.

A simple pin-to-plane electrode arrangement (as described in *Section 2.1.1*) was used for the following tests. The allergen sample was fixed to the earthed, planar electrode and the pin was connected to a d.c. high voltage supply (Model 3807: Alpha Series III, *Brandenberg Ltd.*, Surrey, England). The allergen sample was then exposed to negative Trichel corona with a corona current of 25µA for 120 minutes. The sample was then removed and stored for subsequent assay to determine the concentration of the allergen under test. The control allergen sample was exposed to the ambient atmosphere for each test period, and then also stored for later assessment. All samples were analysed for allergen concentration by two-site monoclonal antibody ELISA.

This protocol was repeated for all the allergens; 30 samples of Der p1, 30 samples of Der f1, 36 samples of Der p2 and 42 samples of Fel d1 were exposed to the corona discharge for 120 minutes per sample. RH was kept constant at 45 ±3% and ambient temperature was constant at 25 ±3°C.

The results were expressed as a mean percentage reduction in allergen concentration, and also as the number of destroyed moles of allergen molecules (see *Section 3.2.1*) in the samples compared to their paired control. The results are shown in *Section 3.10*.

3.2.1 CALCULATION OF THE AMOUNT OF ALLERGEN DESTROYED

Using the concentration data obtained from the results of the ELISA, it was possible to calculate the amount of allergen present in the samples before and after corona discharge. A mole (mol) is the SI unit of amount of substance. It is equal to the amount of substance that contains as many elementary units as there are atoms in 0.012kg of carbon^{12}, i.e., Avogadro's constant (6.0221367 x10^{23}). One mole of a compound has a mass equal to its relative molecular mass expressed in grams.

Der p1 is a 24kDa protein, one Da is equivalent to 1.66033 x10^{-27}kg

$\therefore$ 1 mole of Der p1 = (24000).(1.66033 x10^{-24}g).(6.02214 x10^{23})

= 23996.975g

Dividing the mass of Der p1 in the sample, in grams, by the mass of one mole of Der p1 gave the number of moles present in the sample. The number of moles destroyed was then calculated by subtracting the number of moles in the sample from the number of moles in the control. The values of the destroyed moles of Der p1 were then plotted against the initial Der p1 concentration (i.e., the control concentration).

These calculations were also repeated for: Der p2, a 14kDa protein; Der f1, a 24kDa protein; and Fel d1, a 36kDa protein.

3.3 THE EFFECT OF EXPOSURE TIME ON DER P1, DER F1, DER P2 AND FEL D1

These experiments were performed in order to characterise the response of the different allergens to negative and positive corona discharge over varying exposure times. Previous studies into the effect of surface treatment on polymers have shown that reactions with corona products follow an exponential relationship in respect to time and current (see *Section 1.9*). Oxidation reactions of the polymer surface reach a saturation point where differing reactions counterbalance each other (Laurent, Mayoux, Noel *et al.*, 1983). The relationship between the percentage reduction in allergen concentration (resulting from reactions within the protein molecule) and the duration of exposure to corona discharge will give an indication of the mechanism of allergen destruction by corona discharge. Comparisons between the Group 1 & 2 mite allergens and the major cat allergen Fel d1 will also be possible.

Solutions containing Der p1, Der f1, Der p2 and Fel d1 were prepared according to the protocol outlined in *Section 3.1.* The allergen samples were then prepared by pippetting 100µl onto aluminium foil. The sample and its paired control were prepared adjacently. These were then dried at 37°C. The foil was then cut into 30mm^2 squares with the allergen sample in the centre of each.

A simple pin-to-plane corona apparatus was made (as described in *Section 2.1.1*). Onto this an allergen sample was placed. The pin was connected to a d.c. high voltage supply of a chosen polarity to achieve the desired corona regime. For negative Trichel, ionic bombardment equivalent to a corona current of 25µA was used, for negative continuous glow the current was 90µA. For positive pulse the current was 5µA, and 15µA for positive continuous glow. These values were chosen as being representative of the desired type of corona generated at a constant relative humidity of 45% (see *Section 2.5*). The corona current was maintained at a constant level throughout each test.

The allergen samples were exposed for 1, 15, 30, 45, 60, 120, 240 or 300 minutes. The samples were then removed and stored for subsequent assay to determine the concentration of its principal allergen. The control allergen sample was exposed to the ambient atmosphere for each test period, and then also stored for later assessment. Six replicates were completed for each time exposure. All samples were then analysed for allergen concentration by two-site monoclonal antibody ELISAs.

The above protocol was followed to test the effect of negative Trichel, negative continuous glow, positive pulse and positive glow corona irradiation on Der p1 and negative Trichel and positive glow on Der p2, Der f1 and Fel d1 allergen solutions. The initial concentration of allergen samples was kept constant at approximately 100ngml^{-1}, RH was maintained constant at 45 ±3% and the temperature was constant at 25 ±3°C. The results are shown in *Section 3.11.*

3.4 THE EFFECT OF CURRENT ON DER P1, DER F1, DER P2 AND FEL D1

In *Section 3.3* the effects on Der p1 of negative Trichel, positive pulse and the higher current negative and positive continuous glow corona regimes were investigated over time. No statistical differences were found between the reductions in Der p1 concentration caused by these two regimes (see *Section 3.11*) even though different corona products are reported to be produced in the higher current regimes (see *Section 1.8*). A more detailed investigation into the effect of current on several different allergens is described here. This will allow comparisons to be made with the different mite allergen groups and the cat allergen Fel d1.

Evaporated allergen samples containing approximately 100ngml^{-1} of Der p1, Der f1, Der p2 or Fel d1 were prepared according to the protocol outlined in *Section 3.1*. These were fixed, in turn, onto the planar electrode of the simple pin-to-plane corona apparatus (as described in *Section 2.1.1*). The pin was connected to a d.c. high voltage supply of desired polarity (Model 3807: Alpha Series III, *Brandenberg Ltd.*, Surrey, England). The allergen sample was then exposed for 60 minutes to corona discharge at different corona currents. Corona currents of 2, 5, 10, 25, 30, 40, 50, 80, 90 and 100μA were used with the negative polarity and positive corona currents of 2, 5, 10, 15, 20, 25 and 30μA were also investigated. The corona current was maintained at a constant level throughout each test. The positive currents only ranged from 2-30μA due to the current/voltage characteristics of corona discharge in air with the pin-to-plane electrode spacing used (see *Sections 2.1.2 & 2.5*).

After each exposure, the sample was removed and stored for subsequent assay to determine the concentration of its principal allergen. The control allergen sample was exposed to the ambient atmosphere for each test period, and then also stored for later assessment. Ten replicates were completed for each corona current and polarity. All samples were then analysed for allergen concentration by its appropriate two-site

monoclonal antibody ELISA. RH was constant at 45 ±3% and the temperature was constant at 25 ±3°C. Results are shown in *Section 3.12.*

3.5 THE EFFECT OF RELATIVE HUMIDITY ON THE EFFICACY OF CORONA DISCHARGE TO DESTROY DER P1.

This series of experiments was designed to investigate whether the relative humidity (RH) of the atmosphere surrounding the corona discharge plays a role in the destruction of Der p1. The level of atmospheric humidity is known to affect both the degree of hydration of corona products and also the nature of the species produced (see *Section 1.8*). Differences in the amount of Der p1 destroyed under these conditions could give an indication of the mechanism responsible for corona-destruction of allergens.

Tests were conducted at relative humidities of 2.4%, 10%, 20% and 50%. Atmospheres of 2.4% and 10%RH were achieved using compressed air and colour-indicating silica gel in the experimental set-up described in *Section 2.1.2*. Relative humidities of 20% and 50% were achieved by placing the corona electrodes in a temperature and humidity-controlled room (also described in *Section 2.1.2*). Using the point-to-plane corona electrodes (described in *Section 2.1.1*), samples of evaporated Der p1 solution (prepared according to the protocol outlined in *Section 3.1*) were exposed to negative Trichel (15μA corona current), negative continuous glow (90μA), positive pulse (5μA) or positive glow corona (30μA) at RH levels of 2.4%, 10% and 20%, and 20μA at 50%RH for 120 minutes at each RH level. Controls were placed in the experimental set-up at the same RH for the equivalent duration.

After each exposure, the samples and their controls were placed in an eppendorf and prepared for ELISA to determine the Der p1 concentration. Twelve replicates were performed for each corona regime at each RH value. The results are shown in *Section 3.1.3*.

3.6 THE EFFECT OF MOLECULAR OZONE ON DER P1, DER F1, DER P2 AND FEL D1

Molecular ozone, a powerful oxidizing corona product, was investigated in the experiments described in this section to determine whether it was responsible for the effects of corona discharge on the allergens. Samples of the allergens were exposed to ozone under the experimental conditions present during corona discharge, and also in aqueous solutions of the allergens. Ozone is known to react with the amino acid residues of proteins in solution (Mudd, Leavitt, Ongun *et al.*, 1969) but the effect of this oxidising agent on evaporated samples of proteins has not previously been investigated.

3.6.1 EXPOSURE OF EVAPORATED ALLERGEN SAMPLES TO MOLECULAR OZONE

Solutions containing Der p1, Der f1, Der p2 and Fel d1 were prepared according to the protocol outlined in *Section 3.1.* The evaporated allergen samples were prepared by pippetting 100µl onto aluminium foil. The sample and its paired control were prepared adjacently. These were then dried at 37°C. The foil was then cut into 30mm^2 squares with the allergen sample in the centre of each square.

An ozone production unit (STRG-PC-U-tube, Starna Ind Ltd, UK) was used to generate molecular ozone in an enclosed box connected to a compressed air supply. The ozone was forced out of the unit by the compressed air at a constant rate of 2lmin^{-1}, through the outlet tube (10mm diameter), and onto the allergen sample 10mm below the aperture (see *Figure 3.1*). The ozone concentration, measured using Dräger short-term ozone detecting tubes (*Drager, Sicherheitstechnik GmbH*, Germany), was determined by the rate of airflow and the voltage applied to the unit. Typical ozone concentrations emitted during corona discharge with the single pin-to-plane electrode arrangement described in this thesis were approximately 0.5 – 1ppm. A higher concentration of 50ppm was chosen to clearly determine whether ozone was responsible for the reduction in allergen

concentration observed after exposure to corona discharge. This concentration was achieved by using a flow rate of 2lmin^{-1} and applying 170V to the ozone-generating unit.

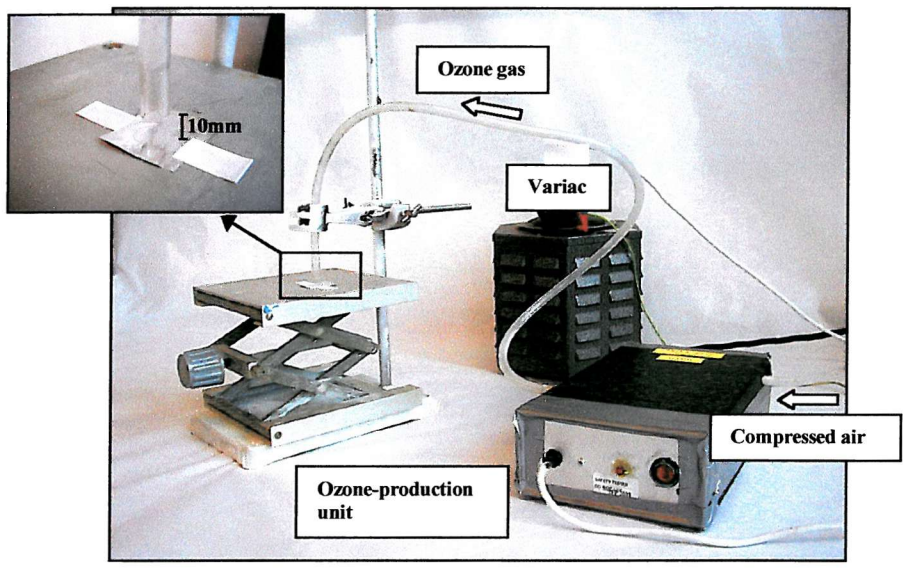


Figure 3.1 **Photographs of the apparatus used to determine the effects of molecular ozone on evaporated allergen samples.**

Following a period of five minutes, in which the concentration of ozone was allowed to stabilise, the foil with the evaporated allergen sample was fixed below the outlet tube and exposed for one hour. After the exposure period, the samples were removed and placed in an eppendorf for later concentration analysis by ELISA. Control samples were later exposed to the air at the same rate of airflow without the ozone production unit in operation. The RH at the allergen sample was 15% and the temperature was 23°C. This protocol was followed in order to expose evaporated samples of Der p1, Der f1, Der p2 and Fel d1. Eight replicates were completed for each allergen. Results are shown in *Section 3.14.*

3.6.2 EXPOSURE OF AQUEOUS ALLERGEN SOLUTIONS TO MOLECULAR OZONE

In order to determine whether ozonolysis destroyed the allergenic proteins, ozone was bubbled through the aqueous allergen solutions. This was achieved by fixing a glass capillary tip with an end aperture of 1mm to the outlet tube (see *Figure 3.2*). This was then lowered into a cuvette containing 500µl of the desired allergen solution, after the ozone concentration had stabilised at 50ppm. After one hour, the pipette was removed and 100µl was removed and stored inside an eppendorf for later concentration analysis by ELISA. This protocol was repeated for the control samples but without the ozone production unit in operation. These were then also placed into an eppendorf for later concentration analysis by ELISA. This protocol was followed for solutions of Der p1, Der f1, Der p2 and Fel d1. Eight replicates were completed for each allergen. Results are shown in *Section 3.14* .

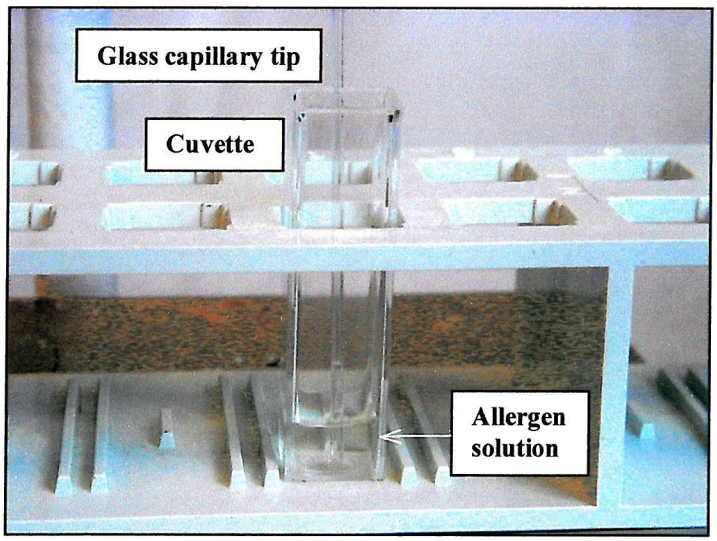


***Figure 3.2* Photograph of the apparatus used to bubble molecular ozone through a sample of aqueous allergen solution.**

3.7 THE EFFECT OF ALUMINIUM PRODUCTS ON DER P1

As described in *Section 1.9*, when aluminium is used as the planar electrode in a pin-to-plane corona set-up, the aluminium does not remain inert but reacts with the corona products to produce a number of aluminium oxides (Goldman, Goldman & Sigmond, 1985). This investigation was performed in order to determine whether the aluminium oxides were responsible for the reduction in Der p1 concentration (as observed in previous sections of this thesis) when a sample of Der p1, evaporated onto aluminium foil, is subjected to corona discharge. The possibility that these oxides affected the integrity of the biological components of ELISA is also explored.

A simple pin-to-plane electrode arrangement was used (as described in *Section 2.2.1*) with a 30mm^2 piece of aluminium foil fixed onto the planar electrode. The pin was connected to a negative d.c. high voltage supply to produce a continuous glow corona discharge with a constant corona current of 90μA. The foil was exposed to the discharge for 120 minutes, and then removed. The central area of the foil, with the layer of aluminium oxides, was carefully removed and placed into an eppendorf with an evaporated Der p1 sample. The Der p1 sample was prepared by pippetting 100μl of Der p1 solution (prepared according to the protocol outlined in *Section 3.1*) onto aluminium foil. The sample and its paired control were prepared adjacently and then dried at 37°C. 500μl of 1% BSA-PBS-T was then added to the Der p1 and foil samples and stored for subsequent assay to determine the Der p1 concentration.

The control 30mm^2 piece of aluminium foil was exposed to the ambient atmosphere for 120 minutes, and then also placed into an eppendorf with an evaporated Der p1 sample and stored in 500μl of 1% BSA-PBS-T for later assessment. This protocol was repeated to produce a total of ten replicates. The Der p1 samples were then analysed for allergen concentration by ELISA. The results are shown in *Section 3.15*.

3.8 INVESTIGATION INTO WHETHER DER P1 CAN RENATURE AFTER EXPOSURE TO CORONA DISCHARGE

This series of experiments was performed in order to determine whether the loss of Der p1 that occurred during corona discharge was temporary. As described in *Section 1.10*, some proteins, after they are denatured, can renature over time. This occurs when only the tertiary structure of the protein has been affected by a change in its environmental conditions. If the protein is returned to optimum conditions it is possible for the conformational structure to return to its natural state. In this series of experiments, samples of Der p1 were exposed to negative corona discharge and then stored in a buffer of optimum pH and assayed for Der p1 after different periods of time. An increase in concentration over time would have indicated that the Der p1 proteins were indeed renaturing.

Using the point-to-plane corona electrodes (described in *Section 2.1.1*), 36 samples of evaporated Der p1 solution (prepared according to the protocol outlined in *Section 3.1*) were exposed to negative continuous glow, corona with a corona current of 90μA, for 60 minutes. Their paired controls were exposed to the ambient atmosphere for the equivalent time. The samples and controls were then placed in an eppendorf. Half the number of samples and their paired controls were left sealed in the eppendorf with nothing added to them, and the 18 other samples and their controls were dissolved into 300μl 0.05% PBS-T. These were all then stored at 4°C.

After 2 weeks storage, 6 samples (and their controls) kept in dry conditions, and 6 samples and controls kept in 300μl 0.05% PBS-T, were prepared for ELISA to determine their Der p1 concentrations. 500μl 1% BSA-PBS-T was added to the samples and controls in dry conditions and 200μl 2.5% BSA-PBS-T was added to the samples kept in 300μl PBS-T. This was repeated after 6 weeks and after 12 weeks storage. The results are shown in *Section 3.16*.

RESULTS AND DISCUSSION

3.9 ALLERGEN CONTENT OF THE AQUEOUS ALLERGEN SOLUTIONS

Figure 3.3 shows the concentration of allergens in the Der p1 solution. This solution contained 101.65 ±1.57ngml^{-1} of Der p1 and also 10.20 ±0.21ngml^{-1} of Der p2. As the solution was made from sieved HDM culture, no Der f1 or Fel d1 allergen was present.

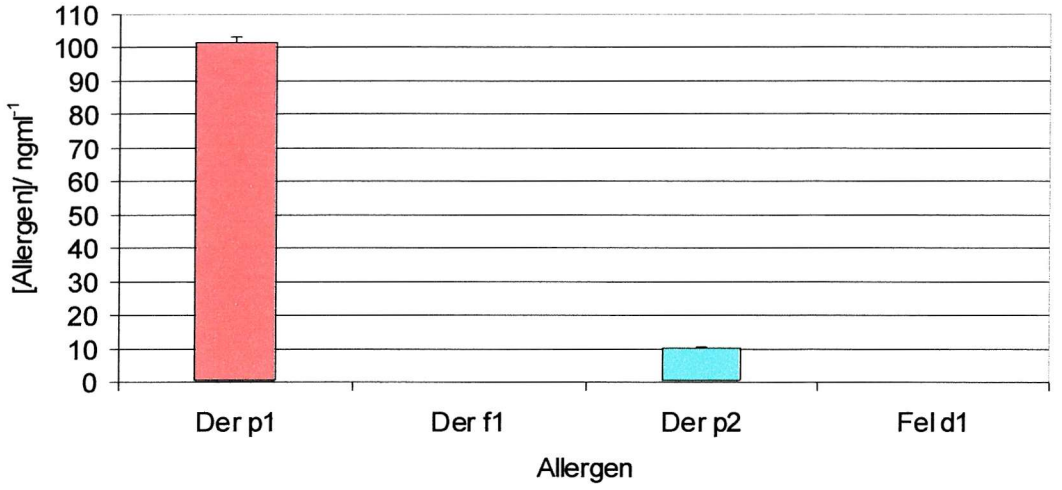


***Figure 3.3* The allergen content of the aqueous 'Der p1 solution'.**
(n=20, SEM shown).

Figure 3.4 shows the concentration of allergens in the Der p2 solution. Der p2 allergen was present at a concentration of 100.44 ±1.18ngml^{-1}. Due to the method of achieving equivalent concentrations of the desired allergen in each solution, and the fact that Der p1 is present in greater concentrations in house dust and cultures (Ford, Rawle, Lind *et al.*, 1985), Der p1 was present in this solution at a concentration of 1006.03 ± 20.67ngml^{-1}. Group 2 allergens have been reported to be present at an approximately five-fold lower concentration than Group 1 allergens in spent mite medium (Ford, Rawle, Lind *et al.*,

1985). However the concentration of Der p2 in this solution was approximately ten-fold less than Der p1. This was probably due to the greater absence of dust mite bodies (~100μm diameter) in the fraction of mite culture used (<63μm). No Der f1 or Fel d1 was detected.

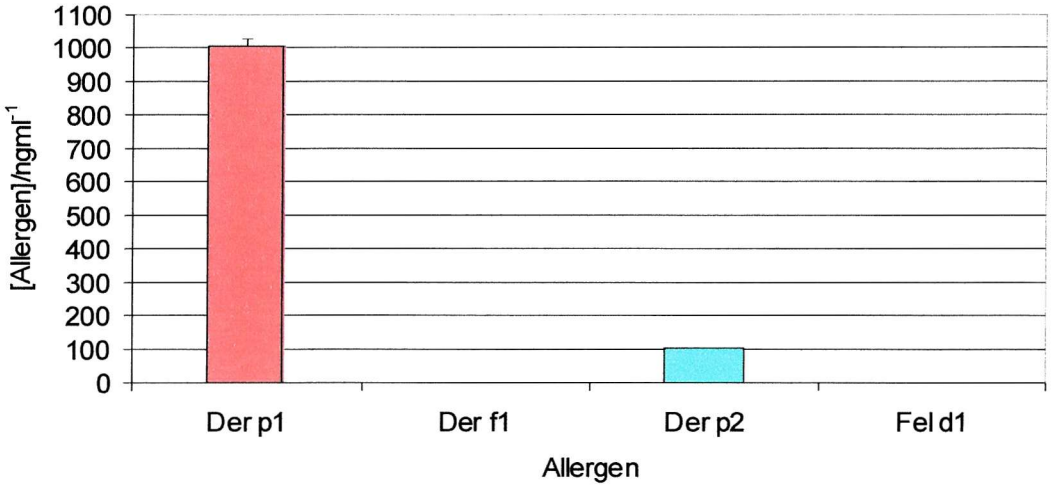


***Figure 3.4* The allergen content of the aqueous 'Der p2 solution'.**
(n=20, SEM shown).

Figure 3.5 shows the concentration of allergens in the Der f1 solution. Der f1 was present in the solution at a concentration of 102.10 ±1.63ngml^{-1}. Der p1 and Fel d1 was also present at concentrations of 5.82 ±1.05ngml^{-1} and 22.69 ±4.49ngml^{-1} respectively. The anti-Group 2 allergen ELISA detected neither Der p2 nor Der f2. *Figure 3.6* shows the concentration of allergens in the Fel d1 solution. Fel d1 was present in the solution at a concentration of 101.42 ±1.29ngml^{-1}. Der p1 and Der p2 was also present in the solution at concentrations of 20.42 ±0.33ngml^{-1} and 2.04 ±0.03ngml^{-1} respectively. No Der f1 was detected in the solution.

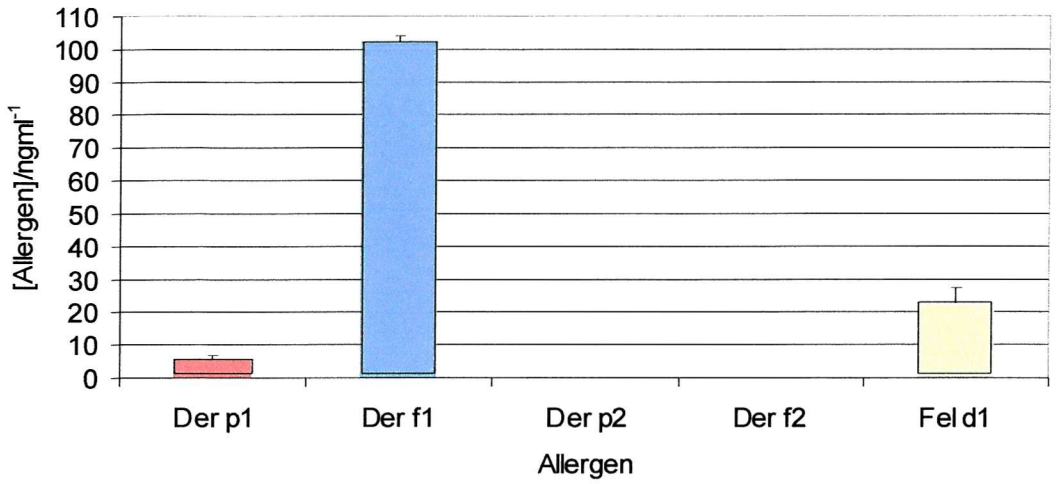


Figure 3.5 **The allergen content of the aqueous 'Der f1 solution'.**
(n=20, SEM shown).

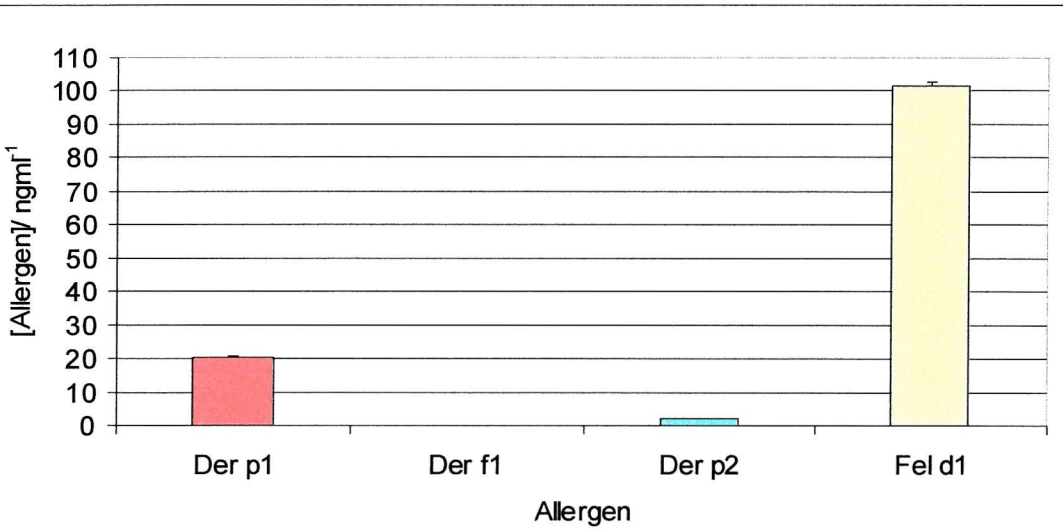


Figure 3.6 **The allergen content of the aqueous 'Fel d1 solution'.**
(n=20, SEM shown).

This analysis shows that although other allergens were present in the solutions prepared to investigate the effect of corona discharge on a single allergen, the desired allergen was

always at a concentration of approximately 100ngml^{-1}. The improved method of achieving concentration homogeneity between samples (investigated in *Sections 2.4 & 2.7*) does not isolate the desired allergen but only gives a reliable value of allergen concentration it contains.

3.10 THE EFFECT OF INITIAL ALLERGEN CONCENTRATION UPON THE EFFICACY OF NEGATIVE CORONA DISCHARGE TO DESTROY DER P1, DER F1, DER P2 AND FEL D1.

DER P1

Figure 3.7 shows the relationship between the initial concentration of Der p1 in the sample (the control concentration) and the mean percentage reduction in Der p1 concentration observed after 120minutes exposure to negative corona discharge. As the initial concentration of Der p1 in the sample increased, the percentage reduction achieved decreased. These reductions fit the exponential decay curve well (R^2=0.957). This moderate, negative correlation was significant (Spearman's rho=-0.672, P<0.01). Under identical experimental conditions, the sample of Der p1 containing 65.37 $ngml^{-1}$ was reduced by 33.78 ± 3.97%. However the sample containing 516.13 $ngml^{-1}$ was reduced by only 7.04 ± 1.75%.

The number of destroyed moles of Der p1 plotted against the starting concentration of Der p1 is shown in *Figure 3.8*. As can be seen, a slight, but significant, positive correlation exists between the two variables (rho=0.461, P<0.05). Therefore, as the initial concentration of the sample was increased, the amount of Der p1 destroyed was also increased. The Kruskal-Wallis test also showed that the data points were statistically different from each other (P<0.05). The overall mean amount of Der p1 destroyed was $1.77 \times 10^{-16} \pm 1.70 \times 10^{-17}$mol ($n$=24).

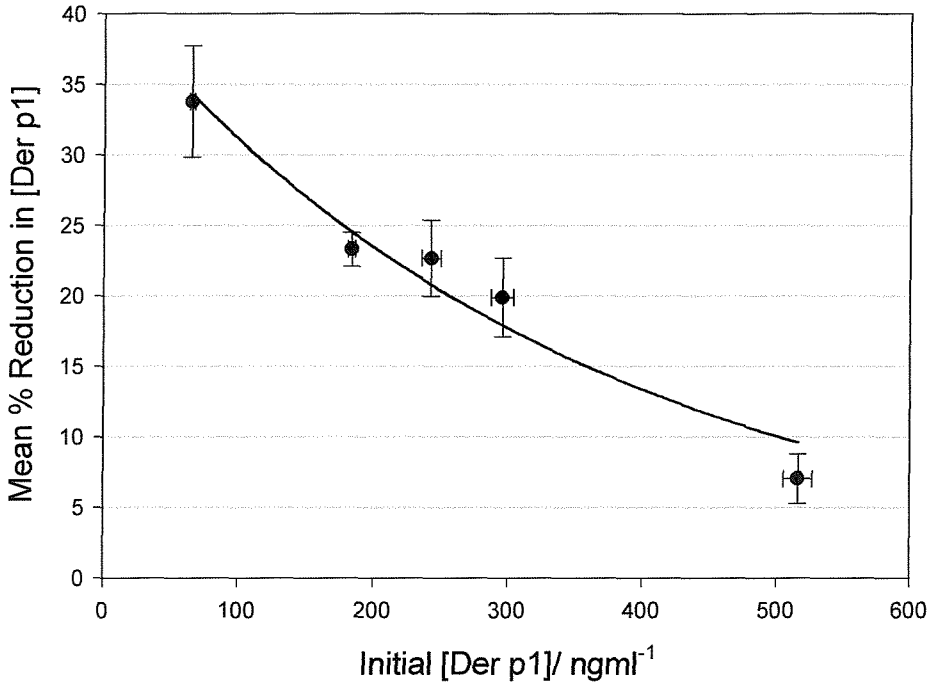


Figure 3.7 **The relationship between the initial concentration of Der p1 in the sample and the mean percentage reduction in Der p1 concentration observed after 120 minutes of negative corona.** (n=6, SEM shown).

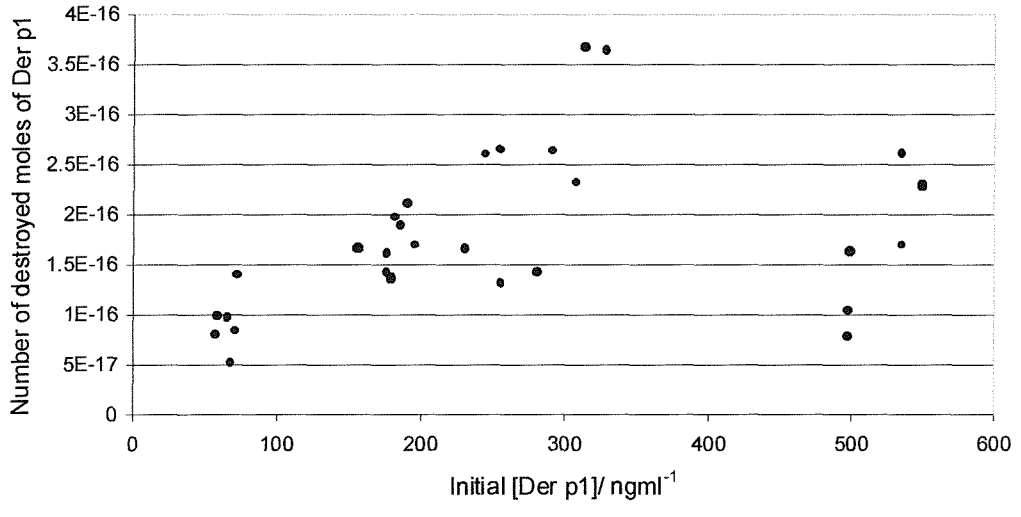


Figure 3.8 **The relationship between the initial concentration of Der p1 in the sample and the number of destroyed moles of Der p1 after 120 minutes of negative corona.**

DER F1

Figure 3.9 shows the statistically significant, modest inverse correlation between the initial Der f1 concentration and the mean percentage reduction in Der f1 (rho=-0.616, $P<0.01$). The sample of Der f1 containing 108.49ngml^{-1} was reduced by 46.84 ± 2.98%, whereas the sample containing 430.64ngml^{-1} was reduced by only 14.40 ± 2.36%. These reductions fit the exponential decay curve well (R^2=0.891).

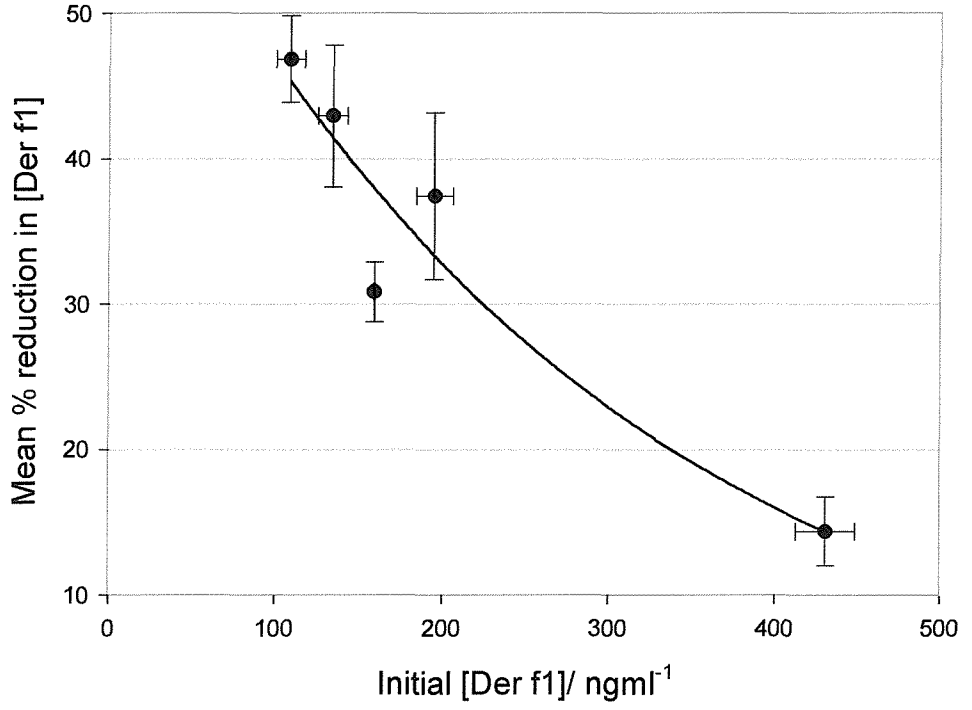


***Figure 3.9* The relationship between the initial concentration of Der f1 in the sample and the mean percentage reduction in Der f1 concentration observed after 120 minutes of negative corona.**

As can be seen in *Figure 3.10*, there is no significant correlation between the number of moles of destroyed Der f1 and the initial sample concentration (rho=0.309, $P>0.05$). The Kruskal-Wallis test showed that there was no statistical difference between the data

(P=0.512). Therefore, the amount of Der f1 destroyed during corona discharge is not dependent upon the initial allergen concentration of the sample. The overall mean was $2.47 \times 10^{-16} \pm 1.75 \times 10^{-17}$mol destroyed per 120min exposure (n=30).

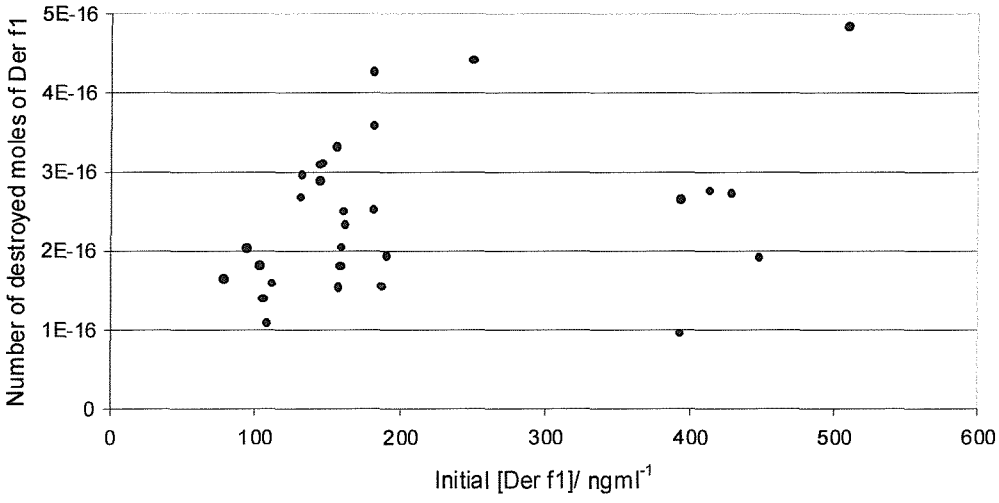


Figure 3.10 **The relationship between the initial concentration of Der f1 in the sample and the number of destroyed moles of Der f1 after 120 minutes of negative corona.**

DER P2

Figure 3.11 shows the highly significant, strong, inverse correlation between the initial Der p2 concentration and the mean percentage reduction in Der p2 concentration (rho=-0.811, P<0.01). The sample of Der p2 containing 42.09ngml^{-1} was reduced by 97.13 $\pm$ 1.05% whereas the sample containing 165.54ngml^{-1} was reduced by only 20.21 $\pm$ 1.71%.

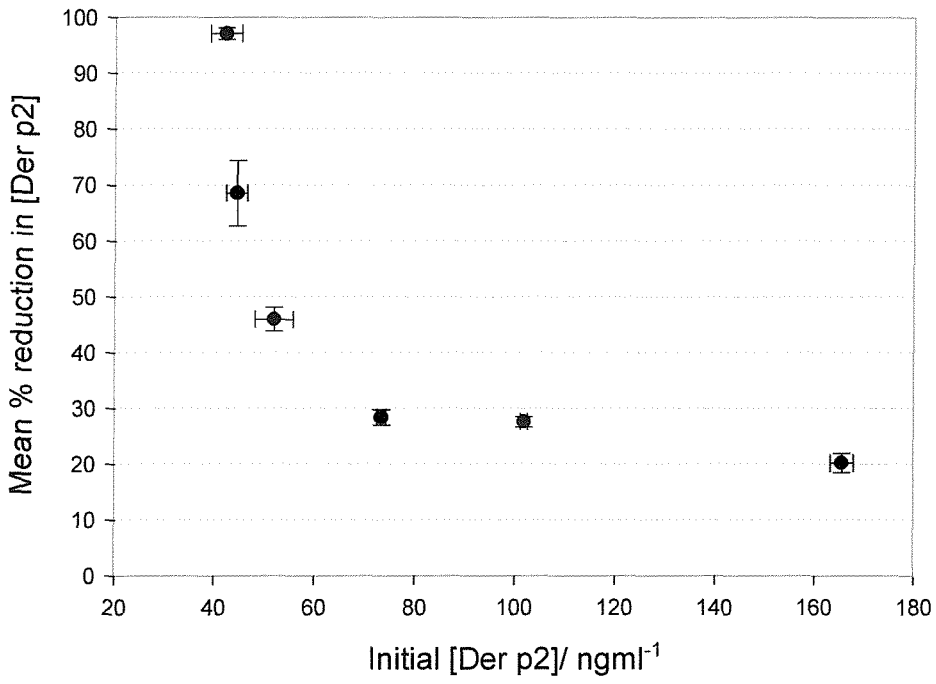


Figure 3.11 **The relationship between the initial concentration of Der p2 in the sample and the mean percentage reduction in Der p2 concentration observed after 120 minutes of negative corona.** (n=6, SEM shown).

As can be seen in *Figure 3.12*, there is no significant correlation between the number of moles of destroyed Der p2 and the initial sample concentration (rho=0.183, P>0.05). However, the Kruskal-Wallis test showed that there was a significant difference between the data points (P=0.004). The overall mean was 2.13 $x10^{-16} \pm 1.13x10^{-17}$mol destroyed per 120min exposure (n=36).

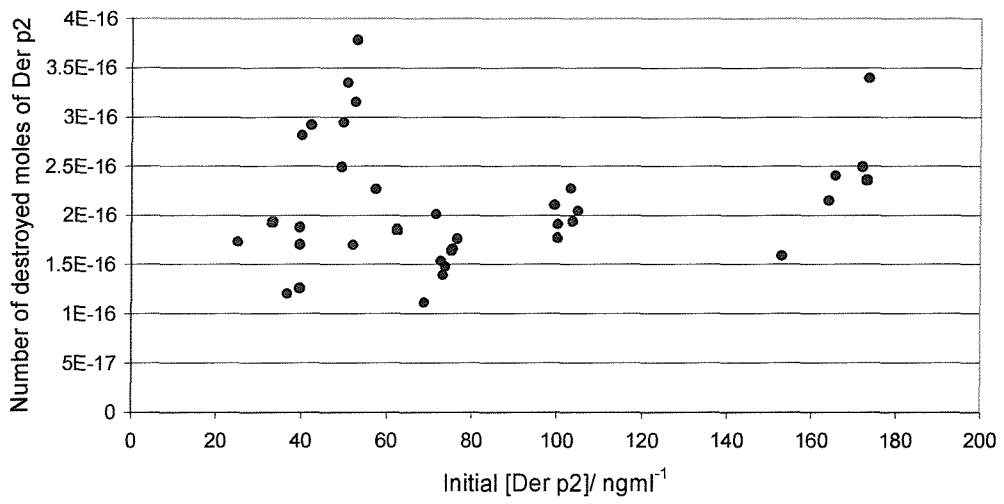


Figure 3.12 **The relationship between the initial concentration of Der p2 in the sample and the number of destroyed moles of Der p2 after 120 minutes of negative corona.**

FEL D1

Figure 3.13 shows the significant, strong, negative correlation between the initial concentration of Fel d1 and the mean percentage reduction in Fel d1 concentration achieved (rho=-0.853, $P<0.01$). The sample containing 247.50ngml^{-1} was reduced by 81.57 ± 1.63% whereas the sample containing 1650.88ngml^{-1} was reduced by only 14.69 ± 1.59%.

As can be seen in *Figure 3.14*, there is no correlation between the number of moles of Fel d1 destroyed and the initial Fel d1 concentration (rho=-0.05, $P=0.976$). However, the Kruskal-Wallis test showed that there was a significant difference between the data points ($P<0.05$). The overall mean was 1.16 x10^{-15} ± 8.79 x10^{-17}mol destroyed per 120 min exposure ($n=42$).

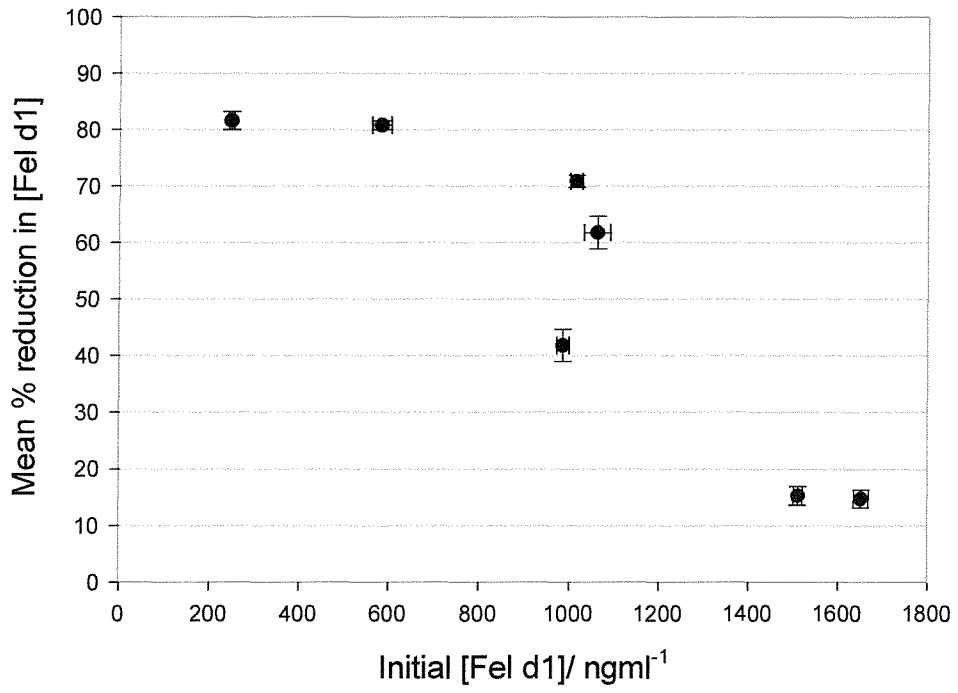


Figure 3.13 **The relationship between the initial concentration of Fel d1 in the sample and the mean percentage reduction in Fel d1 concentration observed after 120 minutes of negative corona.** (*n*=6, SEM shown).

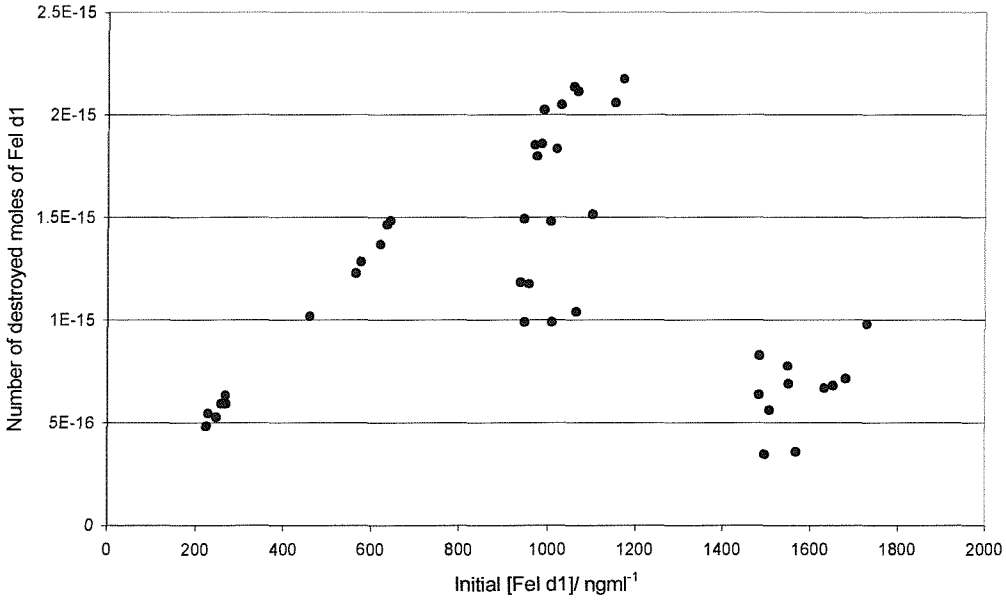


Figure 3.14 **The relationship between the initial concentration of Fel d1 in the sample and the number of destroyed moles of Fel d1 after 120 minutes of negative corona.**

DISCUSSION

All allergens tested showed similar behaviour upon irradiation with corona discharge. A negative correlation existed between the initial concentration of allergen in the sample and the percentage reduction achieved in that sample's concentration. The amount of allergen destroyed per 2 hour exposure with a constant corona current however remained relatively constant. Only Der p1 showed a significant positive correlation between the initial concentration and the number of moles of allergen destroyed, but even this correlation was weak (rho=0.461, $P<0.05$). Although significant differences were found between the number of destroyed moles of allergen in the tests on Der p1, Der p2 and Fel d1, all four of the allergens had similar mean values for the number of moles of allergen destroyed. These can be seen in *Table 3.1*. Compiling the data for all four allergens gave an overall mean of $5.14 \times 10^{-16} \pm 4.76 \times 10^{-17}$mol for the number of destroyed allergens per 2 hour exposure to negative corona discharge with a corona current of 25μA (n=138).

Allergen	Mean Number of Moles destroyed	SEM
Der p1	1.77×10^{-16}	1.70×10^{-17}
Der f1	2.47×10^{-16}	1.75×10^{-17}
Der p2	2.13×10^{-16}	1.13×10^{-17}
Fel d1	1.16×10^{-15}	8.79×10^{-17}
Combined	5.14×10^{-16}	4.76×10^{-17}

***Table 3.1* The mean number of moles destroyed of each allergen investigated.**

Therefore, 'percentage reduction in allergen concentration' cannot be used solely to describe the loss in allergen after corona irradiation; the initial concentration of the sample must also be known. This is because approximately the same amount of allergen is destroyed per unit exposure time. Thus, the fraction of destroyed molecules will be smaller when the initial concentration is greater.

3.11 THE EFFECT OF EXPOSURE TIME ON DER P1, DER F1, DER P2 AND FEL D1

DER P1

Figures 3.15 & 3.16 show the concentration of Der p1 in the samples exposed to the Trichel (25μA corona current), or negative continuous glow regime (90μA corona current) for different exposure times and their paired controls. As the initial concentration of Der p1 was constant, percentage reduction calculations are appropriate to distinguish the effect of corona discharge on the allergen concentration (see *Section 3.10*).

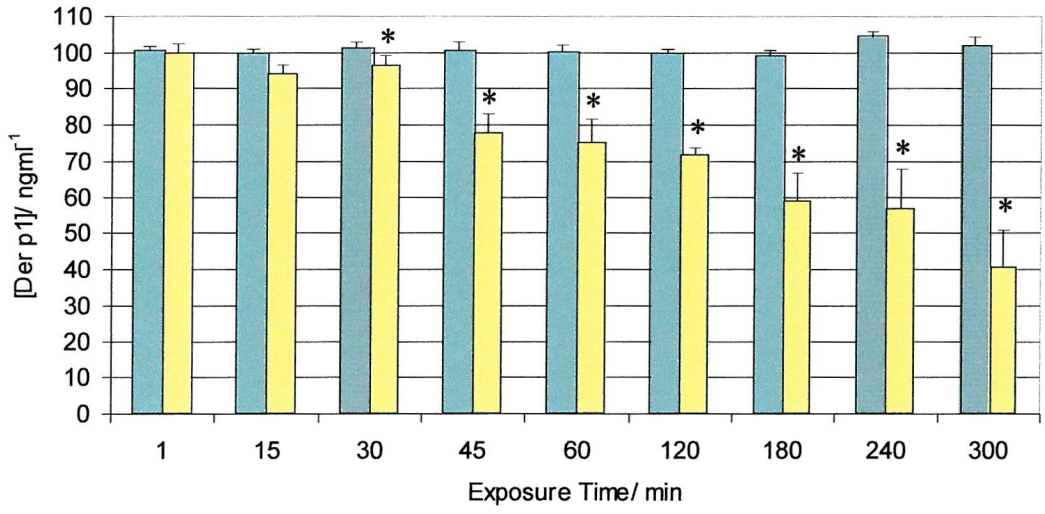


***Figure 3.15* The Der p1 concentrations in the samples exposed to negative Trichel corona and their controls.**
Samples (□) were exposed to 25μA, negative Trichel corona for varying exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

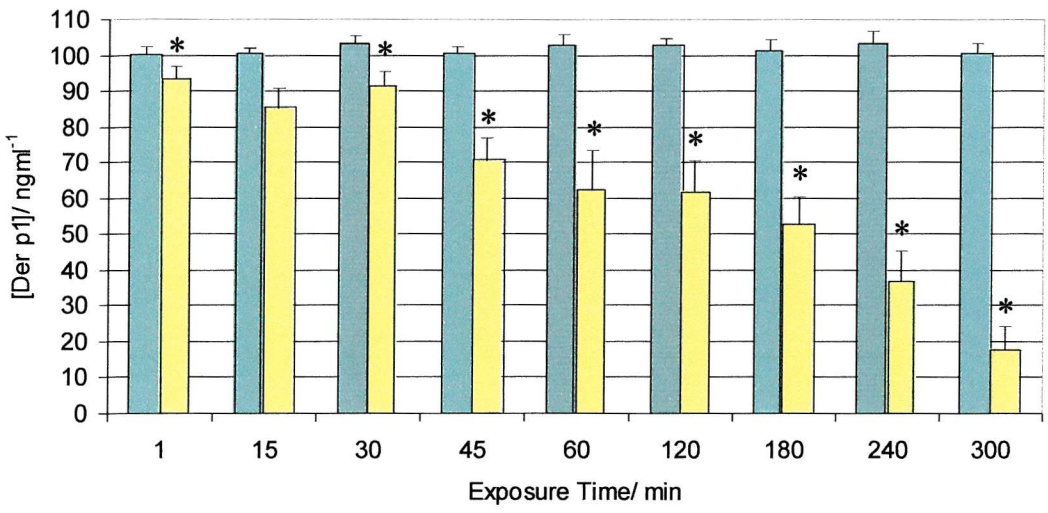


Figure 3.16 **The Der p1 concentrations in the samples exposed to negative continuous glow corona and their controls.**
Samples (□) were exposed to 90μA, negative continuous glow corona for increasing exposure times. Controls (▣) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

Figure 3.17 shows the relationship between the percentage reduction in Der p1 concentration observed after different periods of exposure to either negative Trichel corona, or negative continuous glow corona. A strong, positive correlation between the percentage reductions in Der p1 concentration and the length of exposure to Trichel corona can be seen (Spearman's rho=0.827, P<0.01). The reductions fit the exponential growth to maximum trend line well (R^2=0.885). All reductions were statistically significant after 30 minutes of exposure or more when the concentrations of the samples were compared to their controls using the paired t-test or the Mann-Whitney test where appropriate (P=0.05). These reductions ranged from 0.49 ± 1.77% after one minute to 59.58 ± 10.17% after 300 minutes.

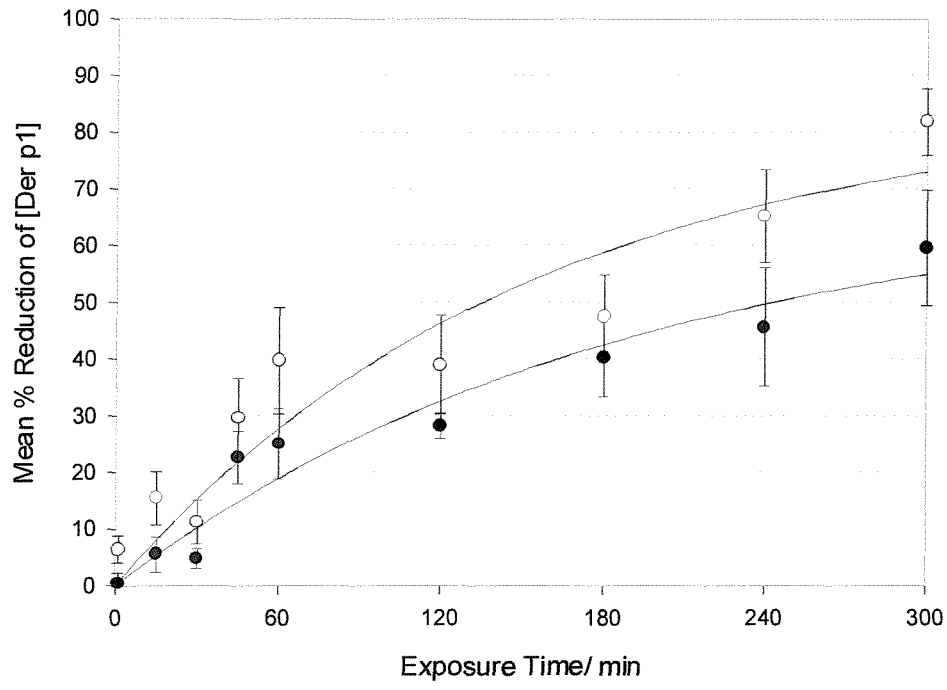


Figure 3.17 **The percentage reduction in Der p1 concentration after exposure to negative continuous glow (○), or negative Trichel (●) corona regimes.**

(n= 6, SEM shown).

A significant, strong positive correlation was also observed between the percentage reductions in Der p1 concentration and the length of exposure time to negative continuous glow corona (rho=0.814, P<0.01). All reductions were statistically significant except after only 15 minutes of exposure (P<0.05) and fit the exponential growth to maximum trend line well (R^2=0.941). Negative continuous glow corona showed a greater efficacy for destroying Der p1 than Trichel, with the percentage reductions ranging from 6.46 ± 2.38% after one minute to 81.76 ± 5.92% after 300 minutes. However, these reductions were not statistically different from those achieved using negative Trichel corona for each exposure period (P>0.05).

Figures 3.18 & 3.19, respectively, show the concentration of Der p1 in the samples exposed to the positive pulse corona (5μA corona current), and the positive continuous glow regime (15μA corona current) for different exposure times and their paired controls.

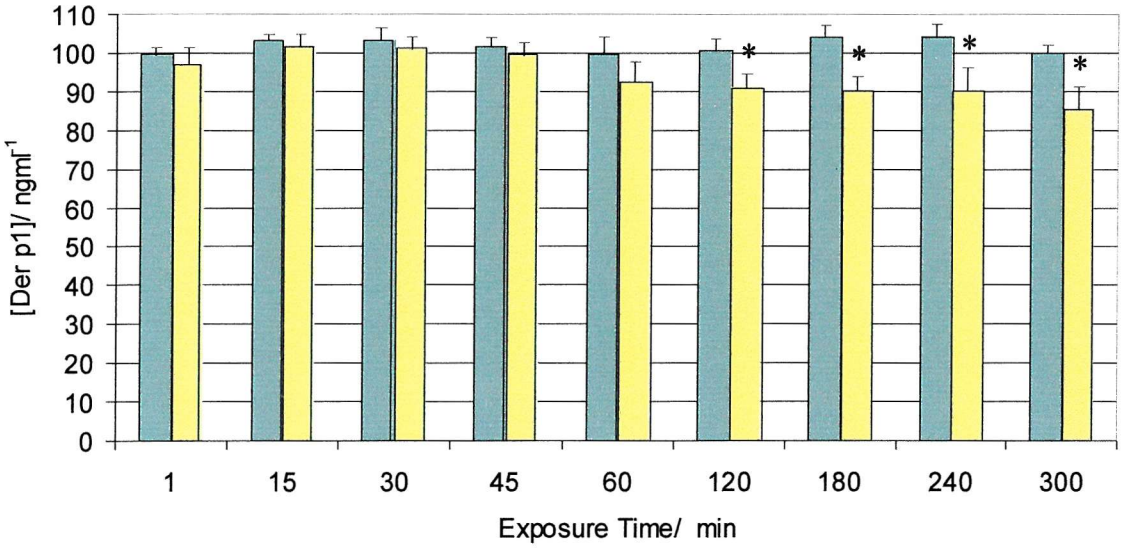


***Figure 3.18* The Der p1 concentrations in the samples exposed to positive pulse corona and their controls.**
Samples (□) were exposed to 5μA, positive pulse corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, *n*=6, SEM shown).

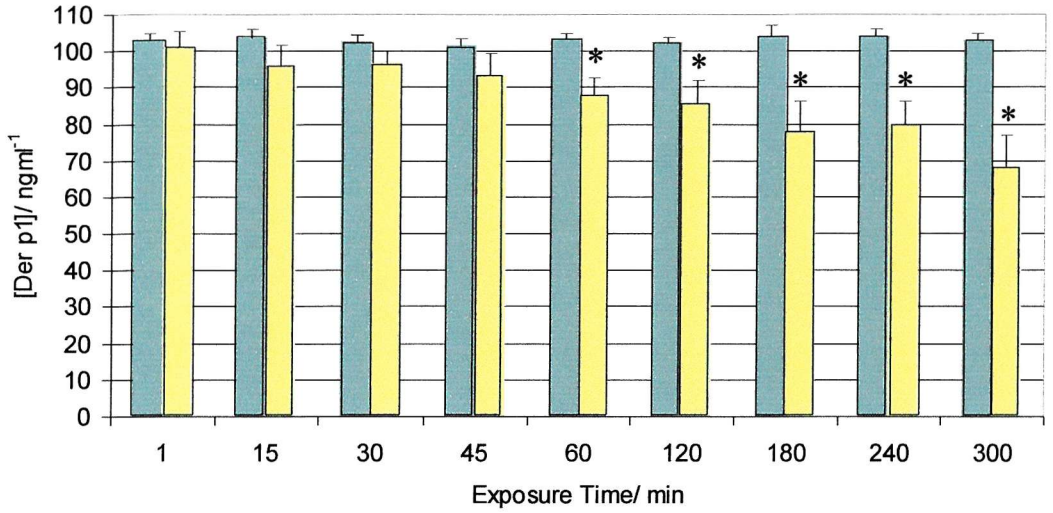


Figure 3.19 **The Der p1 concentrations in the samples exposed to positive continuous glow corona and their controls.**
Samples (□) were exposed to 15μA, positive continuous glow corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

Figure 3.20 shows the relationship between the percentage reductions observed after different periods of exposure to either positive pulse corona, or positive continuous glow corona. Modest positive correlations can be seen between the percentage reductions in Der p1 concentration and the period of exposure to both regimes (rho=0.546 and 0.598, P<0.01 for pulse and continuous glow respectively). These reductions also fit the exponential growth to maximum trend line well (R^2=0.936 and 0.912 for pulse and glow respectively).

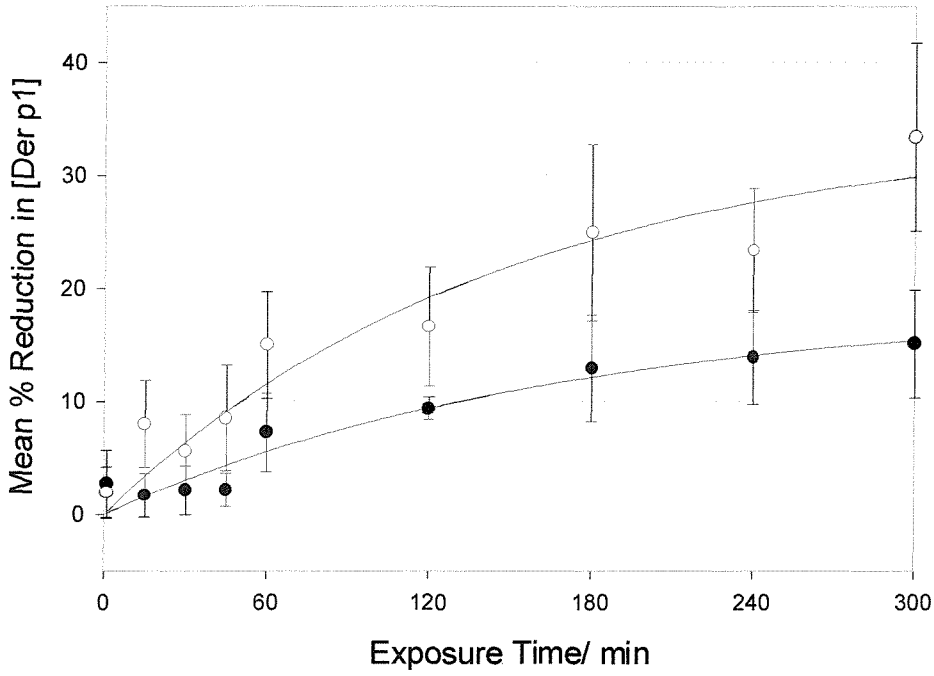


Figure 3.20 **The percentage reduction in Der p1 concentration after exposure to positive continuous glow (○), or positive pulse (●) corona regimes.**

(n= 6, SEM shown).

After exposure to positive pulse corona, reductions were observed that ranged from 2.70 ± 2.96% after one minute to 15.07 ± 4.77% after 300 minutes. All reductions were significant after 120 minutes of exposure or more (P<0.05). As with the negative corona regimes, although not statistically different from those achieved using positive pulse for each exposure period (P>0.05), the reductions observed after exposure to positive continuous glow were greater, ranging from 1.92 ± 2.30% after one minute to 33.41 ± 8.34% after 300 minutes. All reductions were significant after 60 minutes of exposure or more (P<0.05).

These data show that there is no statistical difference in the ability of intra-polarity regimes to reduce Der p1 concentration. As the two regimes within the same polarity cause statistically non-different reductions, the data from both can be combined. *Figure 3.21* shows the relationship between the time of exposure to either negative or positive corona and the percentage reduction of Der p1 concentration. It can be seen that Der p1 concentration was reduced more effectively by negative corona for each time exposure reaching a maximum of 70.67 ± 1.68% after 300 minutes compared with only 24.24 ± 5.35% by positive corona. Exposure to negative corona for 45 minutes or more resulted in reductions that were significantly different from those achieved with positive coronas ($P<0.01$). These curves fit the trend line well (R^2=0.946, 0.952) for negative and positive corona respectively.

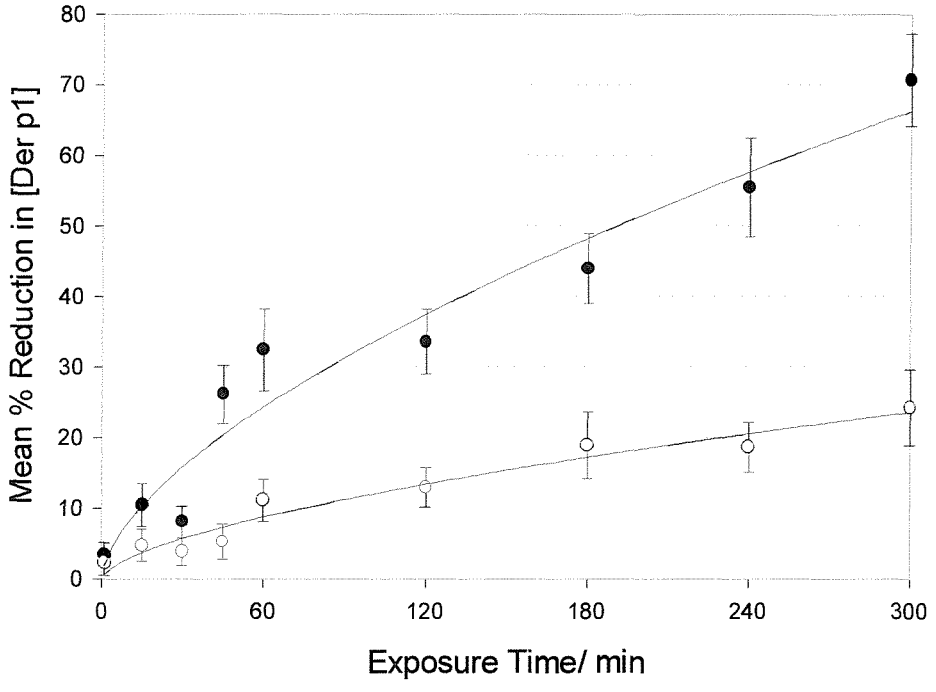


***Figure 3.21* The percentage reduction in Der p1 concentration after exposure to negative (●), or positive (○) corona.**

(n= 12, SEM shown).

Exposure to either negative or positive corona regimes resulted in the reduction of Der p1 concentration. For both polarities, the higher current continuous glow regimes gave a greater mean reduction in Der p1, although the difference between the efficacies of these intra-polarity regimes were not statistically significant. Negative corona types showed a greater efficacy at destroying the Der p1 with a mean percentage reduction 46.43% higher than the greatest positive corona reduction after 300 minutes.

A semi-liquid layer forms on the anodic surface during negative discharge in air at relative humidities (RH) greater than 10% (Goldman, Goldman & Sigmond, 1985). Analysis of the radicals developed in an aqueous solution (see *Section 1.8*) by the arrival of negative ions at the surface, has shown that the species of ions produced is dependent on the regime of the discharge (Lecuiller, Julien & Pucheault, 1972). Although a comprehensive list of ions produced in either negative corona regime is not currently available, from the data presented here it can be seen that no significant difference in the ability of the two regimes' corona products to destroy Der p1 exists.

A recent study of the corona products of the positive regimes has elucidated the different species of ions produced (see *Section 1.8*; Held, Peyrous, 1999). The greater number and variety of ions produced during the continuous glow regime have been shown here not to have significantly greater efficacy at destroying Der p1. The results of a more detailed investigation into the effect of corona currents, ranging from the pulse to the continuous glow regimes, on Der p1, Der f1, Der p2 and Fel d1 is given in *Section 3.12*. A discussion of the possible mechanisms of corona-destruction of the allergens is given at the end of this section.

DER F1

Figures 3.22 & 3.23 show the concentration of Der f1 in the samples exposed to either negative or positive corona for different exposure times and their paired controls. *Figure 3.24* shows the relationship between the percentage reductions observed after different periods of exposure to either negative Trichel or positive glow corona.

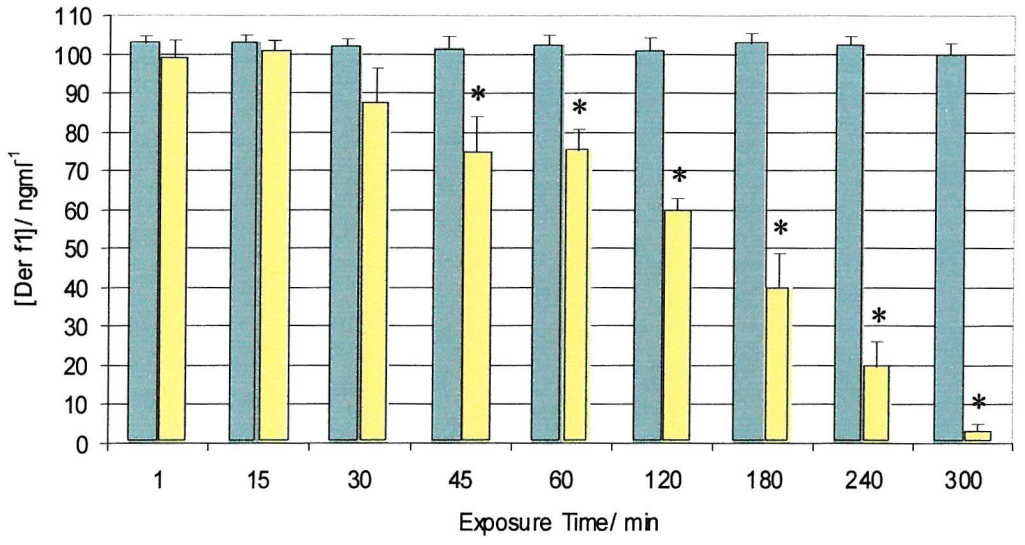


Figure 3.22 **The Der f1 concentrations in the samples exposed to negative Trichel corona and their controls.**
Samples (□) were exposed to 25μA, negative Trichel corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, *n*=6, SEM shown).

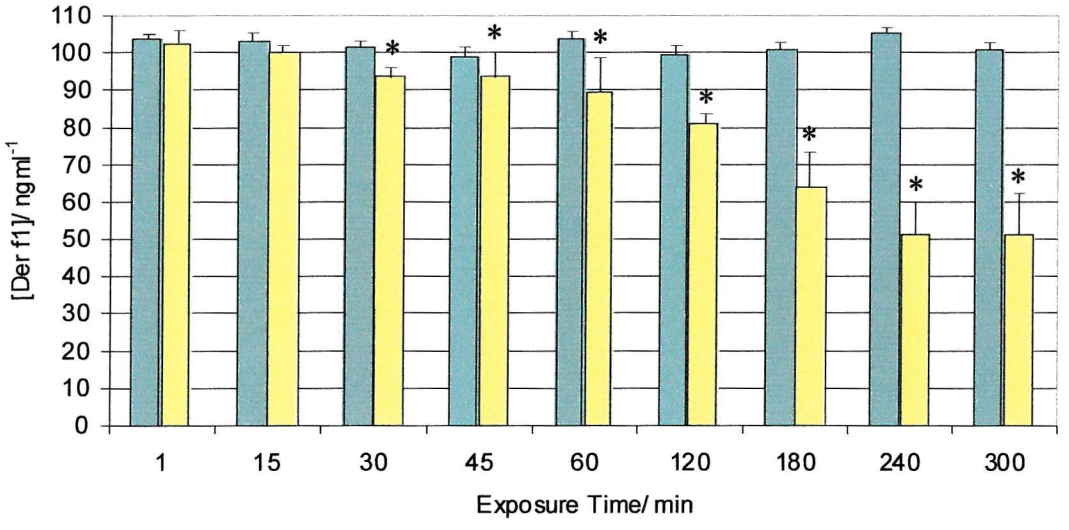


Figure 3.23 **The Der f1 concentrations in the samples exposed to positive continuous glow corona and their controls.**
Samples (□) were exposed to 5μA, positive continuous glow corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, *n*=6, SEM shown).

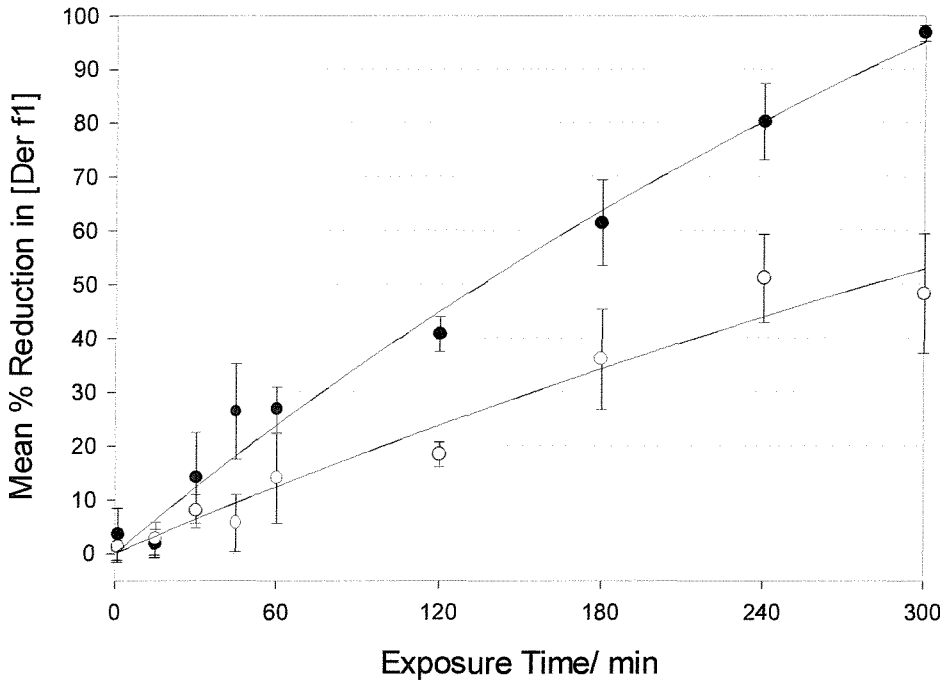


Figure 3.24 **The percentage reduction in Der f1 concentration after exposure to negative (●), or positive (○) corona.**

(n= 6, SEM shown).

Strong, positive correlations can be seen between the percentage reductions in Der f1 concentration and the length of exposure to both corona polarities (rho=0.896 and 0.776, P<0.01 for negative and positive corona exposures respectively). These reductions also fit the exponential growth to maximum trend line well (R^2=0.985, 0.960 for negative and positive corona respectively).

After exposure to negative corona, reductions were observed that ranged from 3.55 ± 4.83% after one minute to 96.70 ± 1.45% after 300 minutes. All reductions were highly significant after 45 minutes of exposure or more (P<0.01). Exposure to positive corona caused reductions that ranged from 1.23 ± 2.82% after one minute, to 48.34 ± 11.12% after 300 minutes. All reductions were significant after 30 minutes of exposure or more

($P<0.05$). Using the Mann Whitney-U test to compare the reductions caused by negative and positive coronas showed that only those reductions caused after 120 and 300 minutes were statistically different from each other ($P<0.05$).

DER P2

Figures 3.25 & 3.26 show the concentration of Der p2 in the samples exposed to either negative or positive corona for different exposure times and their paired controls. *Figure 3.27* shows the relationship between the percentage reductions observed after different periods of exposure to either negative or positive corona.

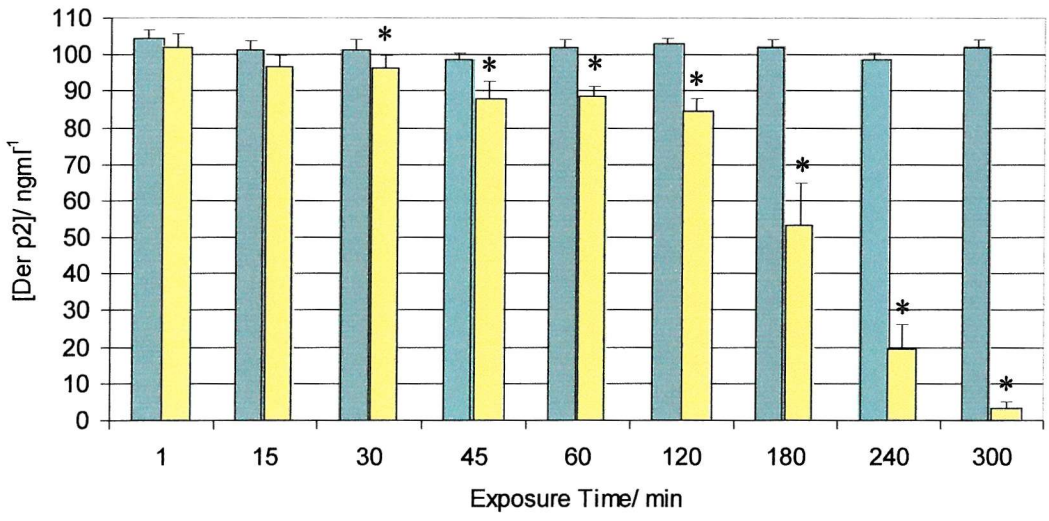


***Figure 3.25* The Der p2 concentrations in the samples exposed to negative Trichel corona and their controls.**
Samples (□) were exposed to 25μA, negative Trichel corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

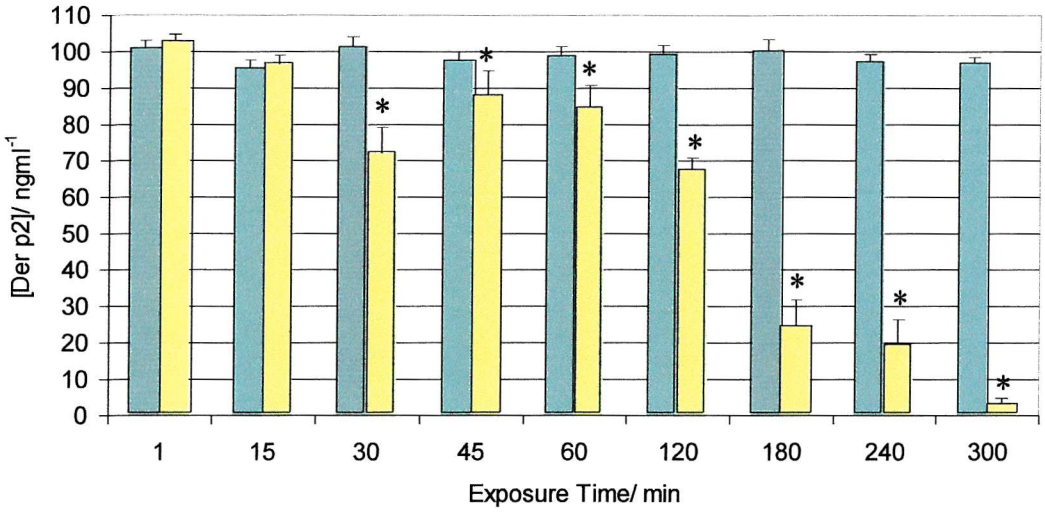


Figure 3.26 **The Der p2 concentrations in the samples exposed to positive continuous glow corona and their controls.**

Samples (□) were exposed to 5μA, positive continuous glow corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, *n*=6, SEM shown).

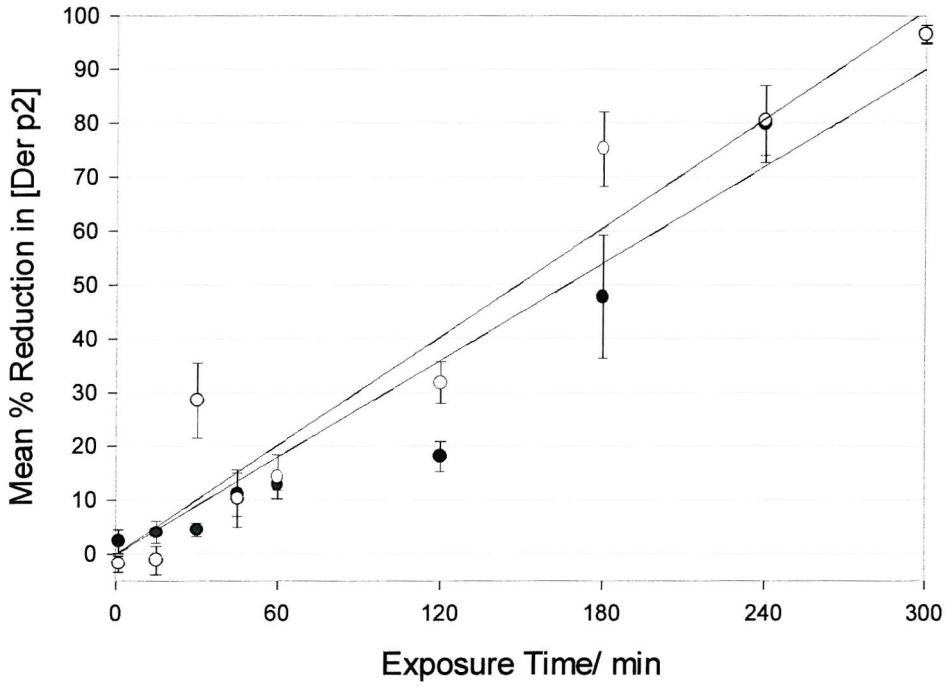


Figure 3.27 **The percentage reduction in Der p2 concentration after exposure to negative (●), or positive (○) corona.**

(*n*= 6, SEM shown).

Unlike the trends observed in all other allergens tested, the percentage reductions in Der p2 concentration are very similar after exposure to negative or positive corona discharge. Using the Mann Whitney-U test to compare the reductions caused by negative and positive coronas showed that only those reductions caused after 30 and 120 minutes were statistically different from each other ($P<0.05$).

Similar, strong, positive correlations can be seen between the percentage reductions in Der p2 concentration and the period of exposure to both corona polarities (rho=0.921, $P<0.01$ for each). These reductions also fit the exponential growth to maximum curve well (R^2=0.948, 0.933 for negative and positive corona respectively). After exposure to negative corona, reductions were observed that ranged from 2.33 ± 2.17% after one minute to 96.57 ± 1.63% after 300 minutes. All reductions were significant after 30 minutes of exposure or more ($P<0.05$). Exposure to positive corona caused reductions that ranged from -1.79 ± 1.48% after one minute, to 96.42 ± 1.69% after 300 minutes. All reductions were significant after 30 minutes of exposure or more ($P<0.05$).

FEL D1

Figures 3.28 & 3.29 show the concentration of Fel d1 in the samples exposed to negative and positive corona respectively for different exposure times and their paired controls. *Figure 3.30* shows the relationship between the percentage reductions observed after different periods of exposure to either negative or positive corona. A modest, positive correlation can be seen between the percentage reductions in Fel d1 concentration and the period of exposure to negative corona (rho=0.672, $P<0.01$). After exposure to negative corona, reductions were observed that ranged from 38.54 ± 5.53% after one minute to 100.00 ± 0.00% after 180 and 240 minutes. All reductions were highly significant ($P<0.01$). The reductions caused by negative corona fit the trend line well (R^2=0.924). The seemingly anomalous result after 300 minutes of exposure, which was only reduced by 92.45 ± 4.19%, is probably due to statistical variation.

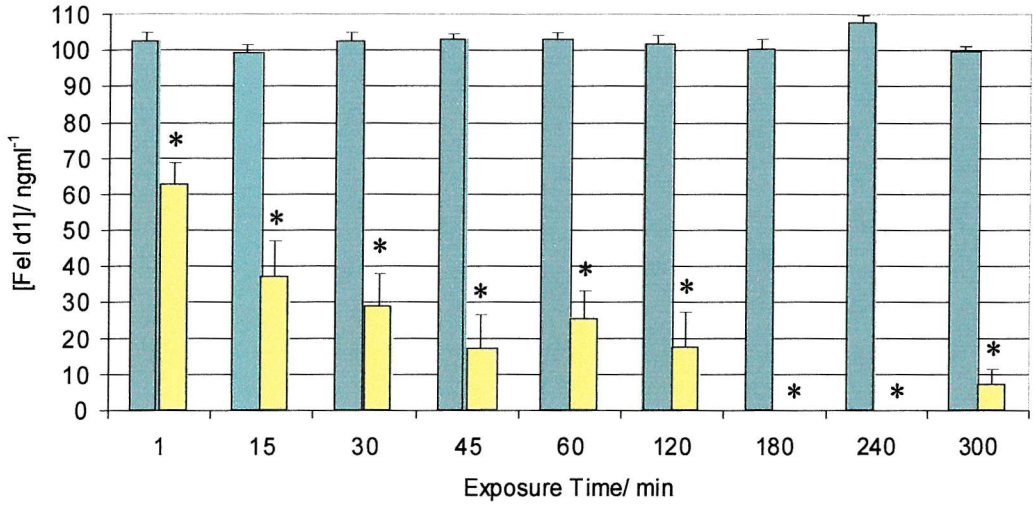


Figure 3.28 **The Fel d1 concentrations in the samples exposed to negative Trichel corona and their controls.**
Samples (□) were exposed to 25μA, negative Trichel corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

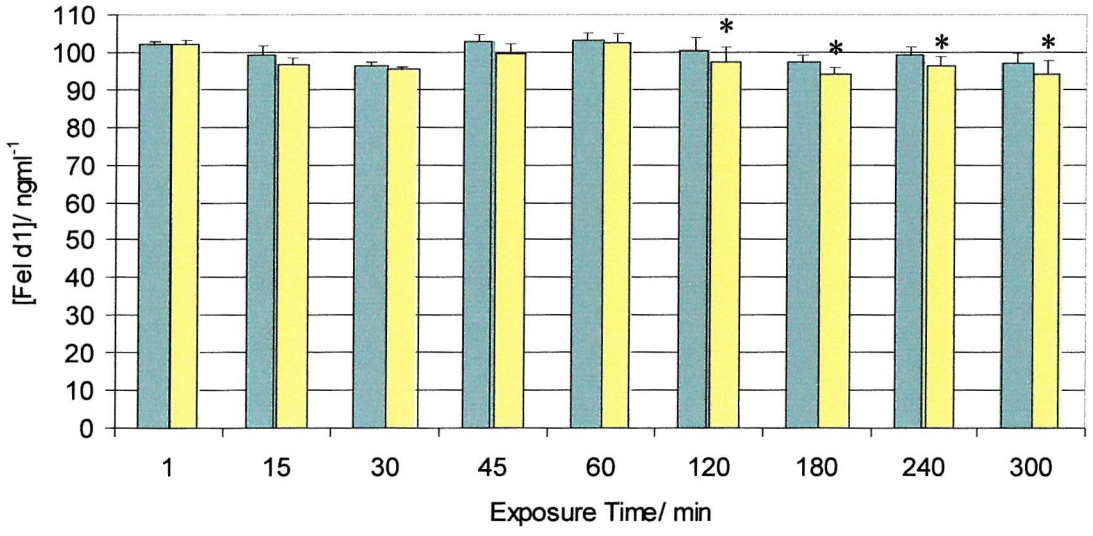


Figure 3.29 **The Fel d1 concentrations in the samples exposed to positive continuous glow corona and their controls.**
Samples (□) were exposed to 5μA, positive continuous glow corona for increasing exposure times. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

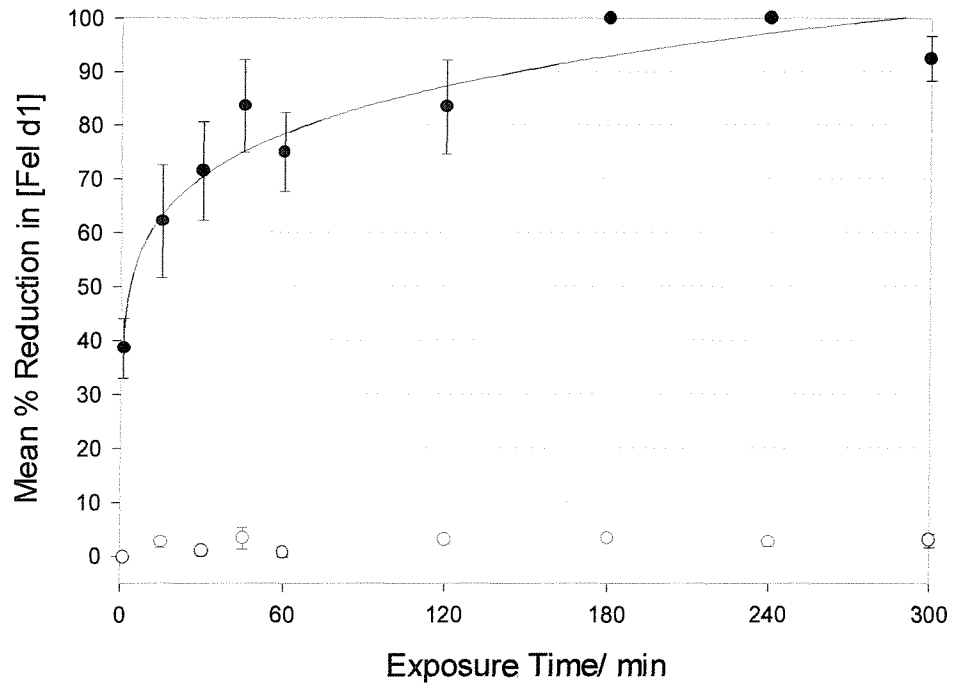


Figure 3.30 **The percentage reduction in Fel d1 concentration after exposure to negative (●), or positive (○) corona.**

(n= 6, SEM shown).

Exposure to positive corona caused very little reduction in the samples' Fel d1 concentration. The percentage reductions observed ranged from –0.18 ± 0.15% after one minute, to 3.27 ± 0.36% after 180 minutes. Only the reductions after 120 minutes or more of exposure were significant (P<0.05). A weak, positive correlation exists between the percentage reductions in Fel d1 concentration and the period of exposure to positive corona (rho=0.333, P=0.014). The Kruskal-Wallis test also showed that the individual reductions were not statistically different from each other (P=0.097). Therefore, the reductions obtained after all time exposures to positive corona can be grouped together as the reduction is not dependent on the length of exposure. The overall mean reduction caused by positive corona was 2.11 ± 0.35% (P<0.01, n=54).

DISCUSSION

Negative corona discharge has been found to destroy all allergens in a time-dependent manner. Positive corona discharge also destroyed all allergens and, except for Fel d1 whose reductions were negligible, all allergens were affected in a time-dependent manner. After exposure to negative corona, Fel d1 had the largest reductions per unit time, after treatment with negative corona, than any other allergen. When compared using the Mann Whitney-U test, these were statistically greater than the other allergens' reductions except after 300 minutes ($P<0.05$).

The reductions observed with Der p1 after negative corona were statistically similar to both Der f1 and Der p2 ($P<0.05$). Thus, the allergen concentrations of the *Dermatophagoides* mite experienced approximately similar reductions after exposure to negative corona, which were all lower per unit time of exposure than those achieved with the Fel d1 allergen.

After exposure to positive glow corona, all *Dermatophagoides* allergens had statistically similar reductions except after an exposure period of 120 minutes or more ($P<0.05$). After this time Der p2 had the greatest reductions per unit exposure time followed by Der f1 and Der p1. The efficacy of positive corona to destroy Fel d1 allergen was very low over all exposure times.

These experiments do not indicate whether the allergen molecules are being chemically modified, cleaved or denatured in the true sense of the term. True denaturing results in the alteration of a protein's tertiary (and/ or quaternary) structure with no change to the protein's primary structure. Although the mechanism of this change is not known, i.e., whether it is due to chemical modification and/ or structural changes, any difference in the molecule would affect its allergenicity. Der p1 is a cysteine protease and its immunogenicity is thought to be reliant on its functionality (Stewart, Thompson & Simpson, 1989), any alteration to its structure would adversely affect its function.

The ELISA results indicate an alteration in the epitopes, which prevents the monoclonal antibody (mAb) from binding. However, as human IgE may recognise different epitopes, the current investigation does not demonstrate that the corona-sensitive epitopes recognised by the monoclonal antibodies are the same as those recognised by the IgE. Further studies are warranted to determine whether the destruction of allergens occurs in the epitopes reacting with human IgE.

The industrial application of corona discharge to treat the surface of polymers was discussed in *Section 1.9*. Some of the bonds encountered in polymers are also present in proteins (which are also polymers), such as the carboxyl group (C-OOH), which will be present at the carboxy terminus of the protein, and CO and NH, which constitute the peptide bond (CONH). During corona discharge treatment of polymers, these bonds are broken and the effect of the corona increases with time and current until a saturation point is reached; then oxidation processes are counterbalanced by decarboxylation processes as revealed by the ejection of CO, CO_2 and H_2 molecules emitted from the surface (Laurent, Mayoux, Noel *et al.*, 1983). This could explain the shape of the exponential growth to maximum curve exhibited by all allergens when subjected to negative or positive corona discharge (except the effect of positive corona on Fel d1).

The maximum percentage reduction obtained by the action of corona discharge on the allergens could represent the saturation point where the oxidation processes were counterbalanced by the decarboxylation processes. Oxidation of the CO, or NH bond, within the peptide bonds, could have lead to the degradation of the protein into its constituent amino acids, which would have been further degraded by the corona products. If this were the case then both the conformational and any linear epitopes would also have been destroyed and there would be no chance of the protein renaturing (see *Section 1.11 & 3.16*).

It has been reported that chemical reduction of Group 1 allergens could possibly affect the disulphide bonds (S-S), thereby unfolding the proteins and giving a more linear conformation. However, when otherwise denatured, by pH or heat, these allergens did not

show this effect (Lombardero, Heymann, Platts-Mills *et al.*, 1990). Der p1 has three disulphide bonds and Der f1 is likely to have a similar number of bonds due to its extensive amino acid homology (Chua, Stewart, Thomas *et al.*, 1988; Kamphuis, Drenth & Baker, 1985). Functionality as enzymes aside, the denaturing of the Group 1 allergens led to a rapid loss of their antigenic activity (Lombardero, Heymann, Platts-Mills *et al.*, 1990). It has been suggested that the antigenic determinants, i.e., the epitopes, of Group 1 allergens are conformational because the loss of antigenic reactivity after reduction and alkylation argues against any strictly sequential epitope sites (Lombardero, Heymann, Platts-Mills *et al.*, 1990). Experiments, using murine mAbs, and sera from mite allergic subjects, have shown that the epitopes of the Group 2 mite allergens are also conformational and that the three disulphide bonds within the protein stabilise this structure (Smith & Chapman, 1996; Lombardero, Heymann, Platts-Mills *et al.*, 1990).

Parallel situations in susceptibility to denaturation have been observed for several other allergens. Virtually any modification of the ragweed allergen Amb a1 makes the molecule essentially non-reactive with IgG or IgE antibodies, and mAbs raised against denatured Amb a1 do not bind the native allergen (King, 1976; Olson & Klapper, 1988; Smith, Olson & Klapper, 1988).

Similar to the chemical reduction of the disulphide bridges leading to a more linear conformation of the Group 1 allergens, as reported by Lombardero *et al* (1990), treatment by corona discharge has also been reported to break these bonds (Demuth, 1984). Under corona discharge, the disulphide bond between cysteine residues is oxidised to form two molecules of cysteic acid, as illustrated in *Figure 3.31*.

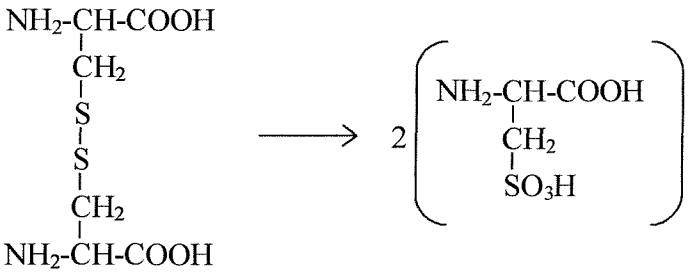


Figure 3.31 **Diagram illustrating the oxidation of the disulphide bond between cysteine residues into cysteic acid under the action of corona discharge.**
The breaking of disulphide bonds within the protein structure would lead to a loss of the protein's precise three-dimensional structure. Based on (Demuth, 1984).

As both Group 1 and Group 2 allergens are likely to contain three disulphide bonds, used to stabilise the conformational epitopes, this reaction of cysteine to cysteic acid may also be responsible for the loss of binding to the mAbs used in ELISA. No data is, at present, available on the presence, or absence, of disulphide bonds in the structure of Fel d1. However similarities between Fel d1 and the Group 2 allergens exist due to their structural stability under heat treatment (Cain, Elderfield, Green *et al.*, 1998).

It is not clear however why Fel d1 was not destroyed to a greater extent under positive glow corona. This exception warrants further research and may shed light on possible structural, or chemical differences between this allergen and the *Dermatophagoides* allergens.

3.12 THE EFFECT OF CURRENT ON DER P1, DER F1, DER P2 AND FEL D1

DER P1

Figure 3.32 shows the concentration of Der p1 in the samples exposed to negative corona at different corona currents and their paired controls. All samples were significantly lower than their paired controls after exposure to corona discharge at currents of 5μA or above, when compared by using either the t-test or the Mann Whitney-U test where appropriate ($P<0.05$). The initial allergen concentration, i.e., the control concentration, was relatively constant at ~100ngml^{-1}. Therefore, the percentage reductions can be validly calculated and used to compare the results at different currents (see *Section 3.10*).

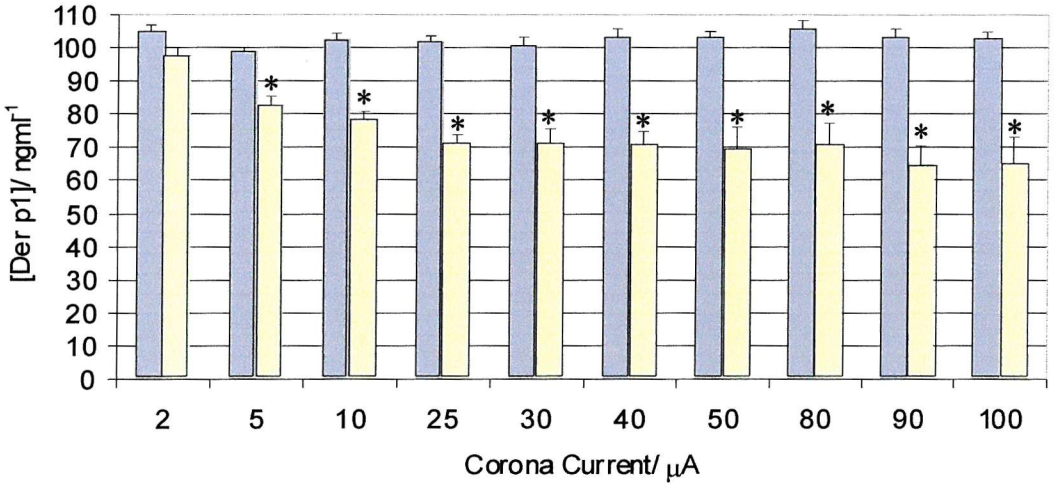


***Figure 3.32* The Der p1 concentrations in the samples exposed to negative corona discharge at different corona currents and their controls.**
Samples (□) were exposed for 60 minutes. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=10, SEM shown).

Figure 3.33 shows the relationship between the percentage reductions in Der p1 concentration observed after exposure to corona discharge at different corona currents. A modest, positive correlation can be seen between the percentage reduction in Der p1 and the current of the corona discharge (rho=0.408, $P<0.01$). After exposure to negative corona, reductions were observed that ranged from 6.91 ± 2.26% at 2μA, to 36.72 ± 7.45% at 100μA. These reductions fit the exponential growth to maximum trend line well ($R^2=0.954$). Reductions increased rapidly from 2μA until 25μA. At corona currents of 60μA or more the trend line shows that reductions reach a plateau at 33.41%.

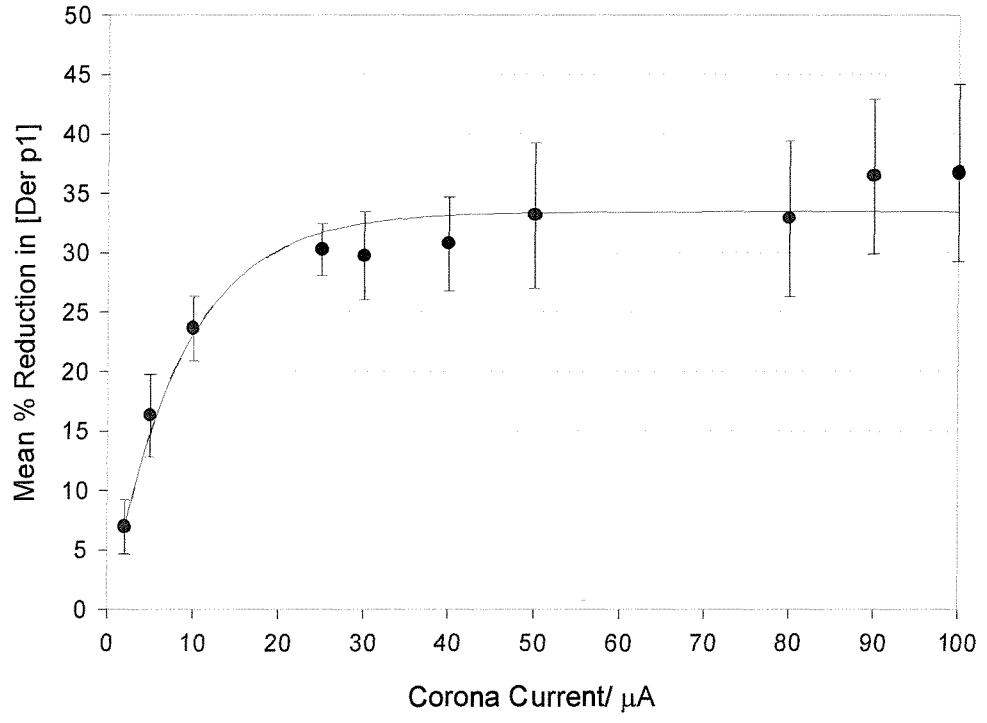


***Figure 3.33* The percentage reduction in Der p1 concentration after exposure to negative corona with different corona currents.**

(*n*= 10, SEM shown).

Figure 3.34 shows the concentration of Der p1 in the samples exposed to positive corona at different currents and their paired controls. All samples were significantly lower in Der p1 concentration than their controls after exposure to corona at 5μA of current or more, which had a constant concentration of ~100ngml^{-1} (P<0.05).

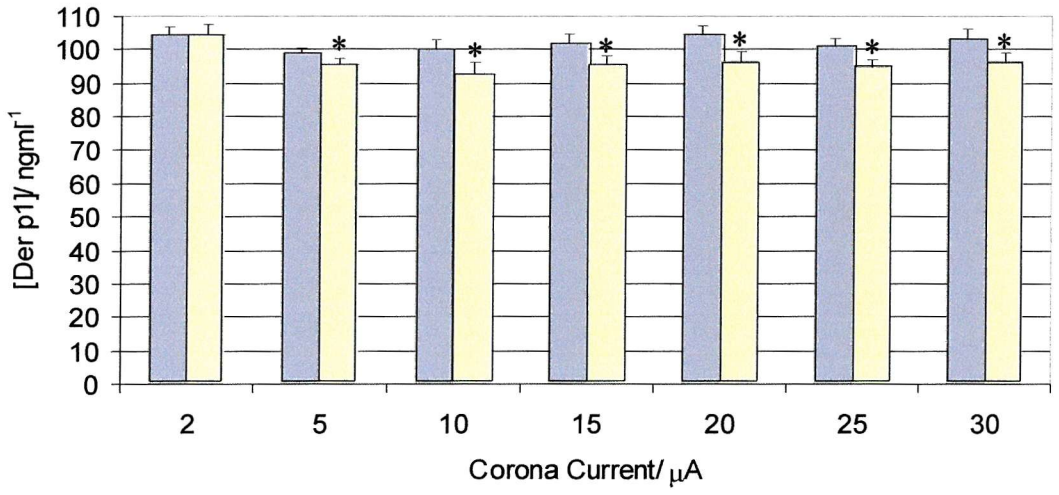


***Figure 3.34* The Der p1 concentrations in the samples exposed to positive corona discharge at different corona currents and their controls.**
Samples (□) were exposed for 60 minutes. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=10, SEM shown).

The weak but significant positive correlation between the percentage reduction in Der p1 and the current of the corona discharge can be seen in *Figure 3.35* (rho=0.380, P<0.01). After exposure to positive corona, small reductions were observed that ranged from –0.14 ± 1.38% at 2μA, to 7.68 ± 1.61% at 20μA, reductions then decreased to 6.47 ± 1.62%. The reductions fit the exponential growth to maximum trend line (R^2=0.815), which shows that reductions would plateau at currents higher than 30μA at approximately 7%.

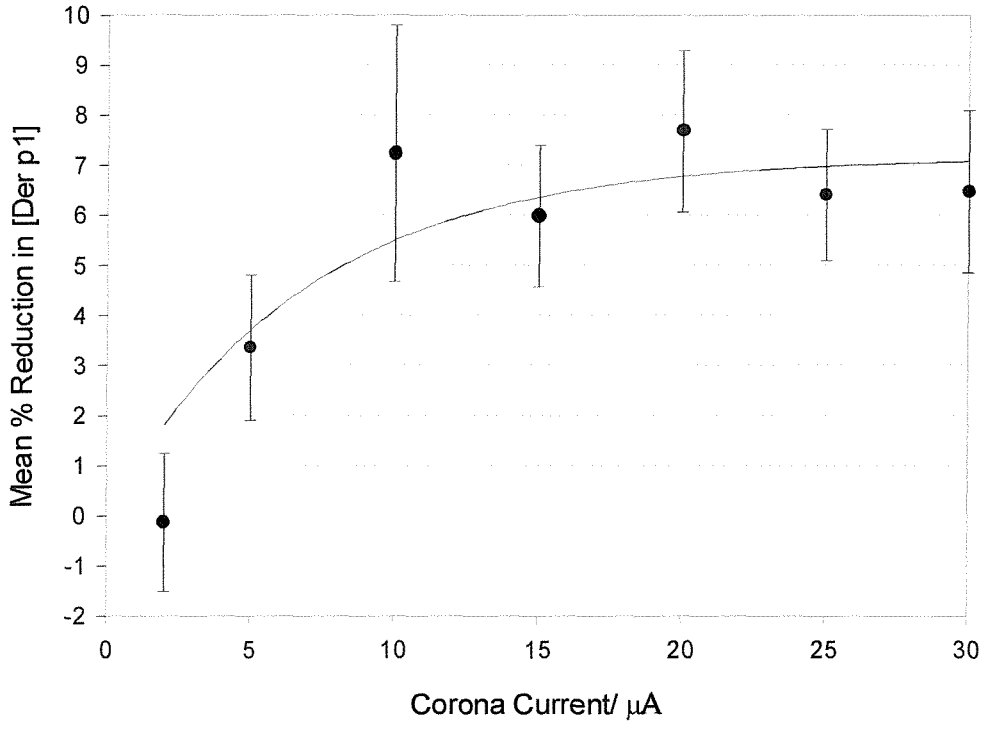


Figure 3.35 **The percentage reduction in Der p1 concentration after exposure to positive corona with different corona currents.**

(n= 10, SEM shown).

DER F1

Figure 3.36 shows the concentration of Der f1 in the samples exposed to negative corona at different corona currents and their paired controls. All samples were significantly lower than their paired controls when compared by either using the t-test or the Mann Whitney-U where appropriate (P<0.05).

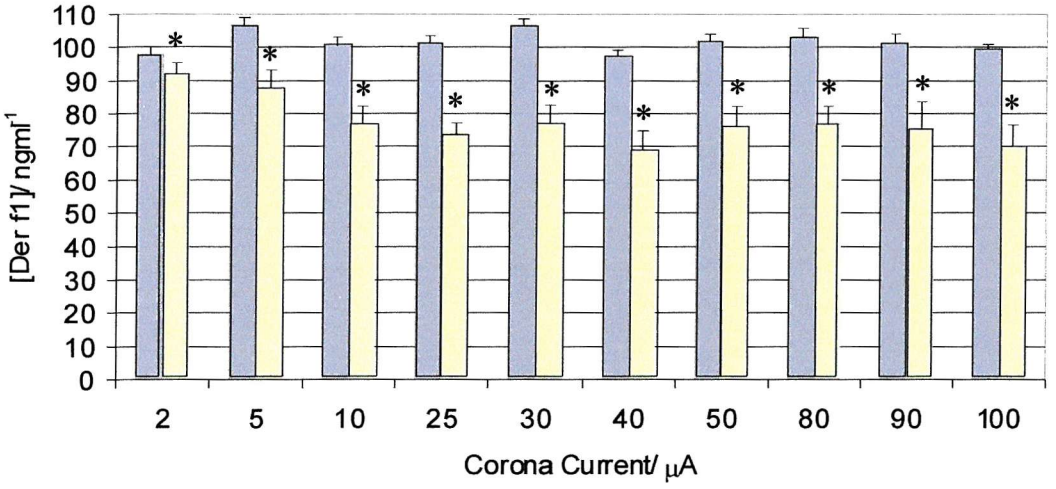


Figure 3.36 **The Der f1 concentrations in the samples exposed to negative corona discharge at different corona currents.**
Samples (□) were exposed for 60 minutes. Controls (▣) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=10, SEM shown).

Figure 3.37 shows the relationship between the percentage reductions in Der f1 concentration observed after exposure to corona discharge at different corona currents. A weak positive correlation can be seen between the percentage reduction in Der f1 and the current of the corona discharge (rho=0.311, P<0.01). After exposure to negative corona, reductions were observed that ranged from 6.10 ± 2.09% at 2μA, to 29.60 ± 6.45% at 100μA. These reductions fit the exponential growth to maximum trend line well (R^2=0.945). Similar to the reductions observed with the Der p1 allergen after exposure to negative corona discharge, reductions increased rapidly from 2μA until 25μA where the reductions remained relatively constant. At corona currents of approximately 45μA or more the trend line shows that the reductions in Der f1 concentration reached a plateau at 27.47%.

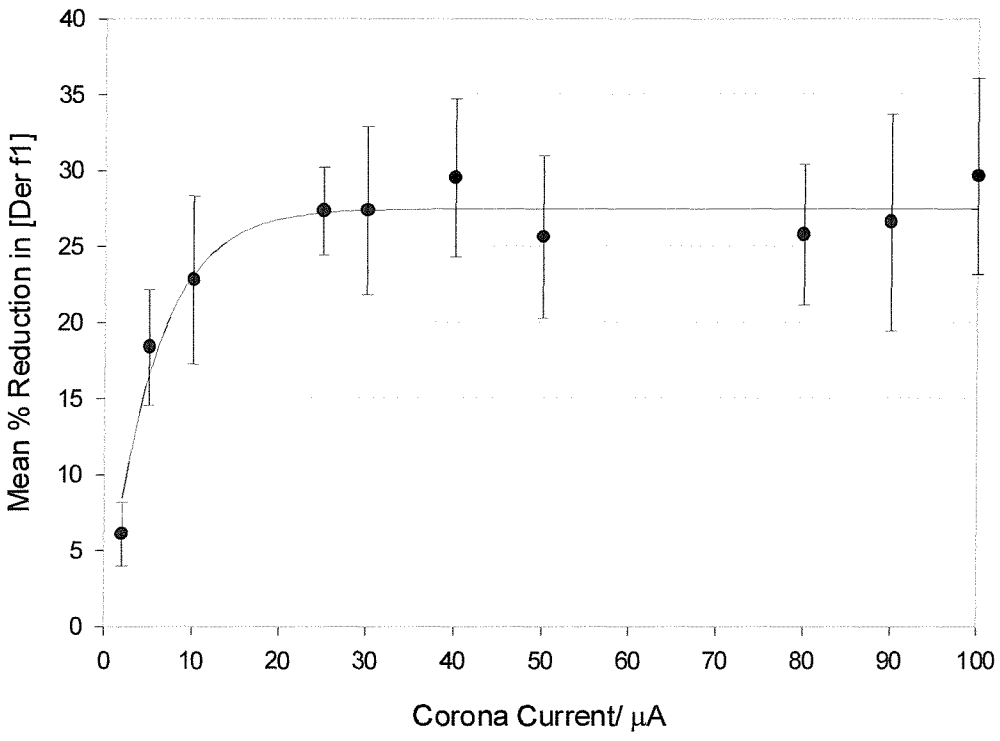


Figure 3.37 **The percentage reduction in Der f1 concentration after exposure to negative corona with different corona currents.**

(n= 10, SEM shown).

Figure 3.38 shows the concentration of Der f1 in the samples exposed to positive corona at different currents and their paired controls. All samples were significantly lower in Der f1 concentration than their controls after exposure to corona currents of 5 μA or more (P<0.05). The weak, but significant, positive correlation between the percentage reduction in Der f1 and the current of the corona discharge can be seen in *Figure 3.39* (rho=0.302, P<0.01). After exposure to positive corona, reductions were observed that ranged from 1.09 ± 2.47% at 2μA, to 10.71 ± 3.24% at 20μA. These reductions fit the exponential growth to maximum trend line well (R^2=0.913) which shows that, although the reductions remained relatively constant after 10 μA or more of corona current, a plateau would be reached at corona currents higher than 30μA at approximately 10.5%.

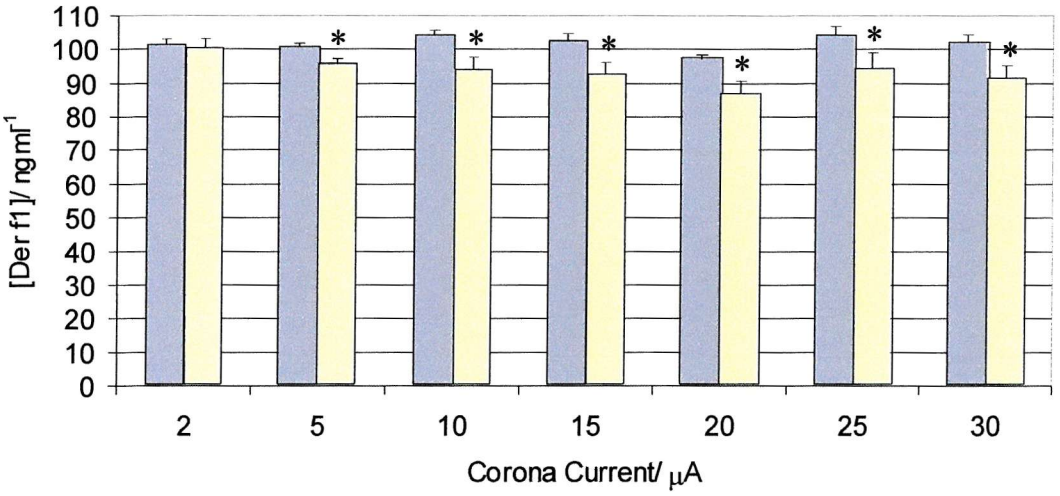


Figure 3.38 **The Der f1 concentrations in the samples exposed to positive corona discharge at different corona currents and their controls.**
Samples (□) were exposed for 60 minutes. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, *n*=10, SEM shown).

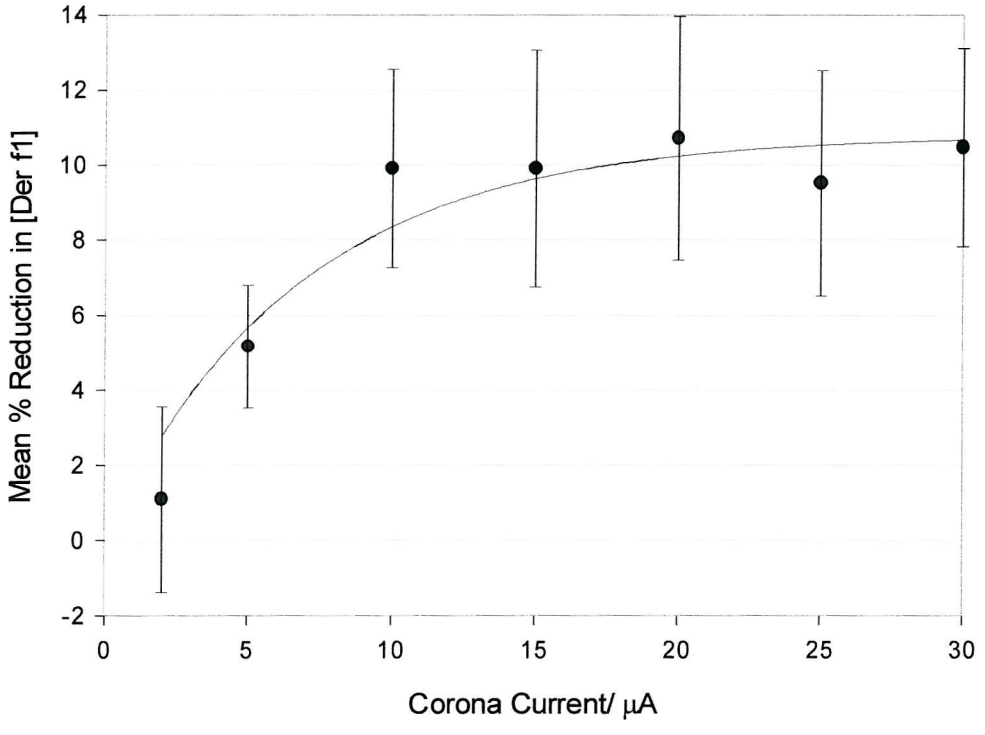


Figure 3.39 **The percentage reduction in Der f1 concentration after exposure to positive corona with different corona currents.**

(*n*= 10, SEM shown).

DER P2

Figure 3.40 shows the concentration of Der p2 in the samples exposed to negative corona discharge at different corona currents and their paired controls. All samples were significantly lower than their paired controls after exposure to corona discharge at currents of 5μA or above, when compared by either using the t-test or the Mann Whitney-U where appropriate ($P<0.01$).

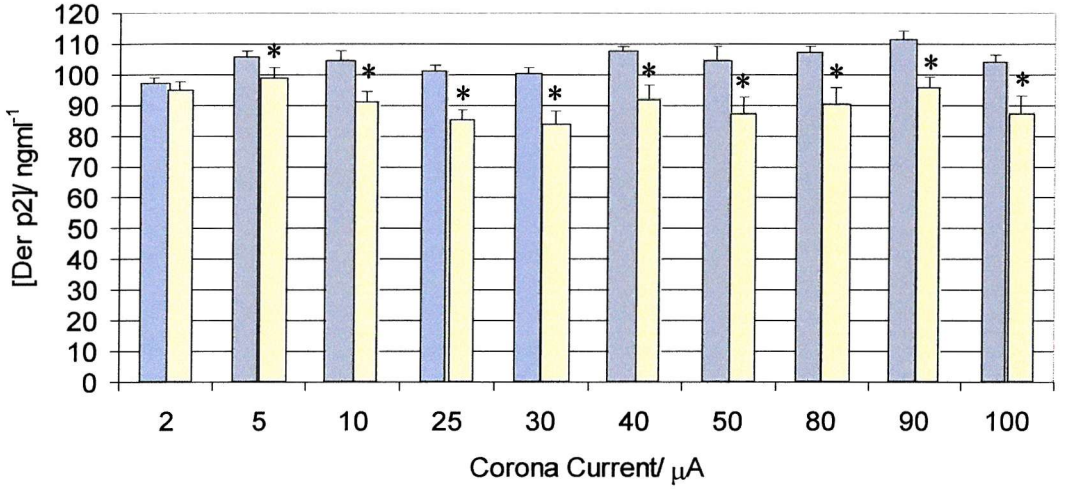


***Figure 3.40* The Der p2 concentrations in the samples exposed to negative continuous glow discharge at different corona currents.**
Samples (□) were exposed for 60 minutes. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=10, SEM shown).

Figure 3.41 shows the relationship between the percentage reductions in Der p2 concentration observed after exposure to corona discharge at different corona currents. A weak positive correlation can be seen between the percentage reduction in Der p2 and the current of the corona discharge (rho=0.302, $P<0.01$). After exposure to negative corona, reductions were observed that ranged from the insignificant 2.02 ± 1.91% at 2μA, to 16.12 ± 4.39% at 50μA. These reductions fit the exponential growth to maximum trend

line well (R^2=0.942). Reductions increased rapidly from 2μA until 25μA where reductions remained relatively constant. The trend line shows that the reductions reached a plateau at 15.50% at corona currents of approximately 45μA or more.

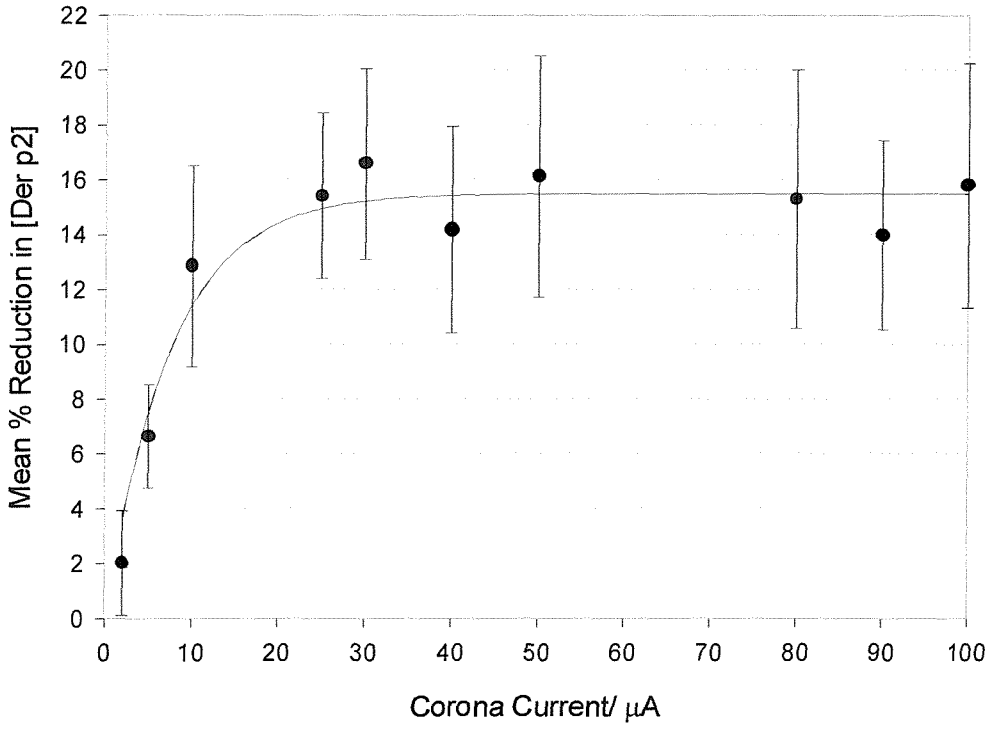


Figure 3.41 **The percentage reduction in Der p2 concentration after exposure to negative corona with different corona currents.**

(*n*= 10, SEM shown).

Figure 3.42 shows the concentration of Der p2 in the samples exposed to positive corona at different currents and their paired controls. All samples were significantly lower in Der p1 concentration than their controls after exposure to corona at 5 μA of current or more, which had a constant concentration of ~100ngml^{-1} (P<0.05).

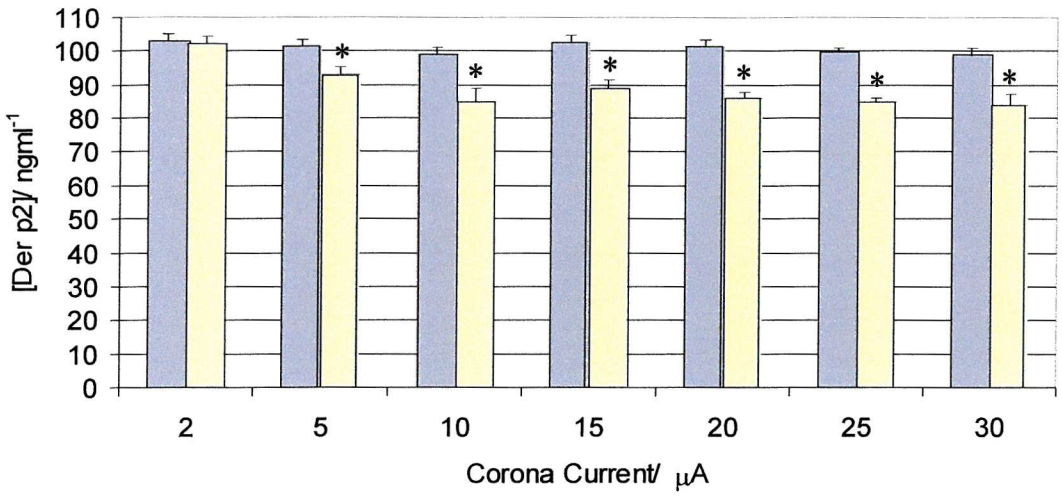


Figure 3.42 **The Der p2 concentrations in the samples exposed to positive corona discharge at different corona currents.**
Samples (□) were exposed for 60 minutes. Controls (▣) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=10, SEM shown).

The significant, modest, positive correlation between the percentage reduction in Der p2 and the current of the corona discharge can be seen in *Figure 3.43* (rho=0.518, P<0.01). After exposure to positive corona, small reductions were observed that ranged from 0.64 ± 0.36% at 2μA, to 15.09 ± 2.01% at 20μA, reductions then remained relatively constant. These reductions fit the exponential growth to maximum trend line (R^2=0.888), which shows that at corona currents higher than 30μA a plateau would be reached at approximately 15.4%.

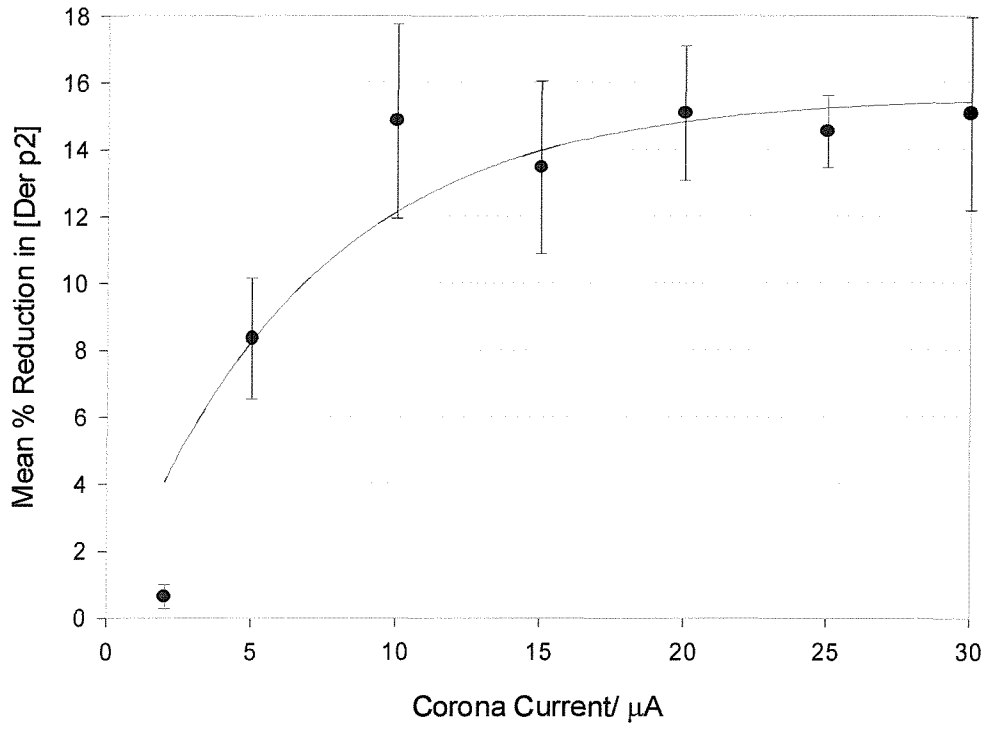


Figure 3.43 **The percentage reduction in Der p2 concentration after exposure to positive corona with different corona currents.**

(n= 10, SEM shown).

FEL D1

Figure 3.44 shows the concentration of Fel d1 in the samples exposed to negative corona discharge at different corona currents and their paired controls. All samples were significantly lower than their paired controls after exposure to corona discharge at all currents, when compared by either using the t-test or the Mann Whitney-U where appropriate (P<0.01).

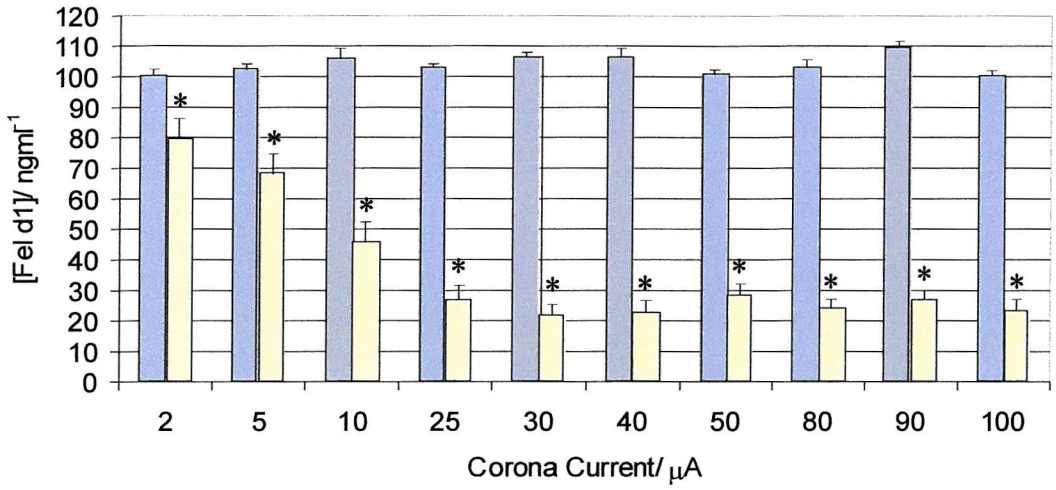


Figure 3.44 **The Fel d1 concentrations in the samples exposed to negative corona discharge at different corona currents.**
Samples (□) were exposed to negative corona discharge at different corona currents for 60 minutes. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=10, SEM shown).

Figure 3.45 shows the relationship between the percentage reductions in Fel d1 concentration observed after exposure to corona discharge at different corona currents. A modest, positive correlation can be seen between the percentage reduction in Fel d1 and the current of the corona discharge (rho=0.551, P<0.01). After exposure to negative corona, reductions were observed that ranged from 20.65 ± 6.93% at 2μA, to 79.08 ± 3.28% at 30μA. At currents higher than 30μA, a slight but statistically insignificant decrease in reductions can be seen. These reductions fit the exponential growth to maximum trend line very well (R^2=0.983). Reductions increased rapidly from 2μA until 25μA where they remained relatively constant. The trend line shows that at approximately 50μA of corona current a plateau was reached at 76.48% reduction. These percentage reductions in Fel d1 concentration were the largest reductions observed for all the allergens tested.

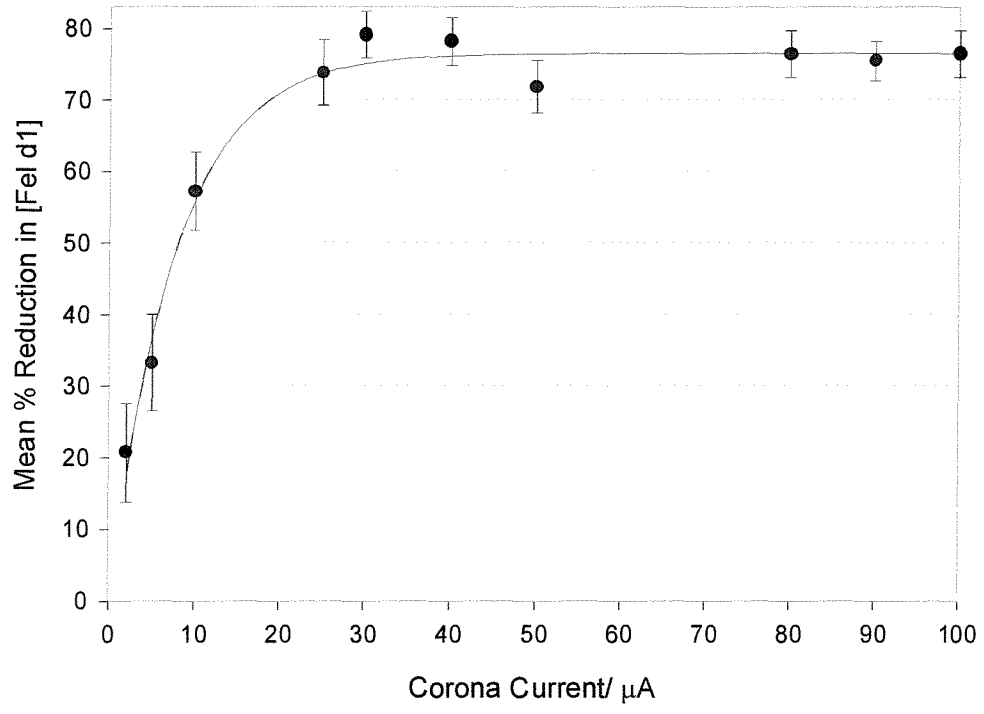


Figure 3.45 **The percentage reduction in Fel d1 concentration after exposure to negative corona with different corona currents.**

(n= 10, SEM shown).

Figure 3.46 shows the concentration of Fel d1 in the samples exposed to positive corona at different currents and their paired controls. No samples were significantly reduced in Fel d1 concentration after exposure to any corona current for the exposure time of 60 minutes. All concentrations were approximately 100ngml^{-1}.

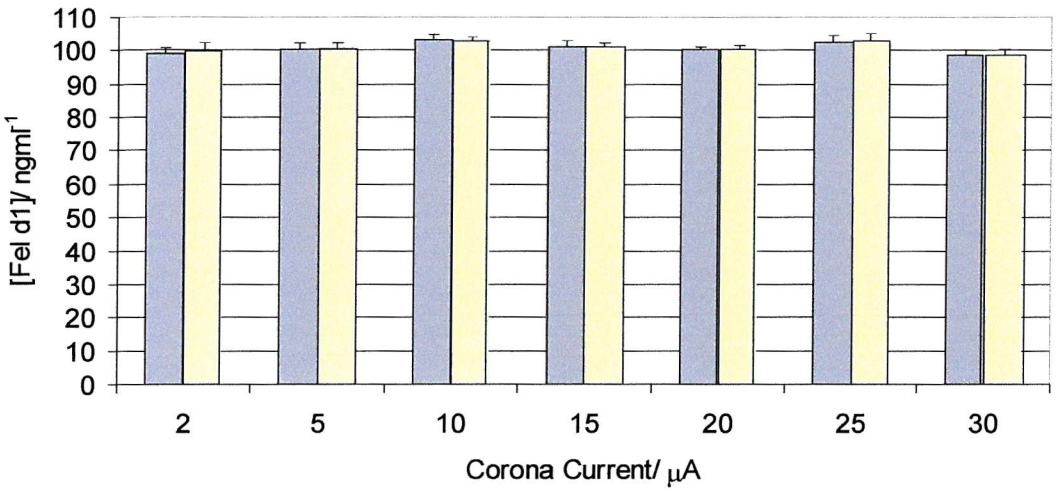


Figure 3.46 **The Fel d1 concentrations in the samples exposed to positive corona discharge at different corona currents.**
Samples (□) were exposed for 60 minutes. Controls (▣) were exposed to the ambient atmosphere for the equivalent period (no samples were statistically lower than their control, n=10, SEM shown).

DISCUSSION

It has been shown that the magnitude of the corona current affects the corona's efficacy to destroy the allergen under exposure. The Fel d1 allergen had the greatest reductions after exposure to negative corona. These reductions were all statistically higher than the reductions observed in the *Dermatophagoides* allergens at negative currents greater then 5μA when compared using the Mann Whitney-U test. After the exposure time of 60 minutes, only Fel d1 was not significantly reduced at any positive current tested. This result was also found during the investigation into the effect of exposure time on the allergens (see *Section 3.3 & 3.11*) and may indicate a difference in the structure of the allergen, which enables it to remain stable after exposure to positive corona discharge.

Statistically similar reductions in allergen concentration were found with Der p1 and Der f1 for each current value of both polarities. This is probably due to Der f1's 81% primary sequence homology to Der p1 (see *Section 1.5.1;* Miyamoto, Oshiwa, Mizimo *et al.*,

1969; Smith, Disney, Williams *et al.*, 1969; Bilotti, Passaleva, Romagani *et al.*, 1972). The reductions observed with the Der f1 and Der p2 allergens were also similar at each current value and polarity. Analysis revealed that these two allergens gave statistically different reductions at only 5 and 25μA, which is due only to statistical variation in the sample population. The reductions in Der p1 and Der p2 concentration caused by corona of both polarities were statistically different from each other at currents of 5μA and above.

The reductions in Der p2 concentration caused by exposure to either polarity were statistically similar to each other. This was also found during the investigation into the effect of exposure time on the Der p2 allergen (see *Section 3.11*). The efficacy of corona to destroy this allergen is the same regardless of the polarity.

As discussed in *Section 3.11*, some of the bonds encountered in polymers are also present in proteins, such as the carboxyl group (C-OOH), which will be present at the carboxy terminus of the protein, and the CO and NH bonds, which constitutes the peptide bond (CONH). During corona discharge treatment of polymers, the above bonds are broken proportional to the amount of exposure time and also the corona current until a saturation point is reached. This is due to the oxidation processes becoming counterbalanced by decarboxylation processes as revealed by ejection of CO, CO_2 and H_2 molecules emitted from the surface (Laurent, Mayoux, Noel *et al.*, 1983). This may also explain the exponential growth to maximum relationship of the percentage reduction in allergen concentration exhibited by all allergens (except positive corona on Fel d1) when subjected to negative or positive corona discharge of increasing corona current. The maximum percentage reduction plateau may represent the saturation point where the oxidation processes were counterbalanced by the decarboxylation processes.

3.13 THE EFFECT OF RELATIVE HUMIDITY ON THE EFFICACY OF CORONA DISCHARGE TO DESTROY DER P1

The results of this investigation show that negative and positive coronas have different dependences upon the atmospheric humidity for their allergen-reducing efficacies. *Figure 3.47* shows the Der p1 concentrations of the samples exposed to negative and positive corona discharge at 2.4%RH. It can be seen that the concentration of the samples exposed to positive corona at this low RH were statistically lower than their paired controls when compared using either the paired t-test for means or the Mann Whitney-U test as appropriate ($P<0.01$). The samples exposed to negative corona experienced no such reduction. This difference in the ability of negative and positive corona to destroy Der p1 may suggest that the two polarities use different reaction mechanisms to reduce the allergen content of the samples.

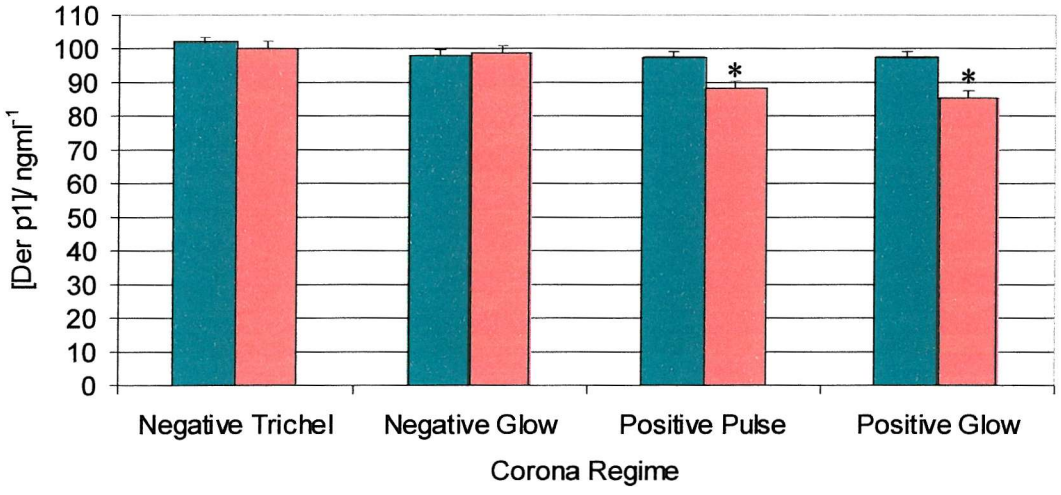


***Figure 3.47*. The Der p1 concentrations in the samples exposed to corona discharge at 2.4%RH and their controls.**
* = Samples (■) statistically lower than their controls (■), $n = 12$, SEM shown.

At higher RH values of 10%, 20% and 50%, the samples exposed to both negative and positive corona were statistically lower in concentration than their paired controls ($P<0.01$); see *Figures 3.48 – 3.50.* The concentration of the samples after exposure to each corona regime was similar at each relative humidity.

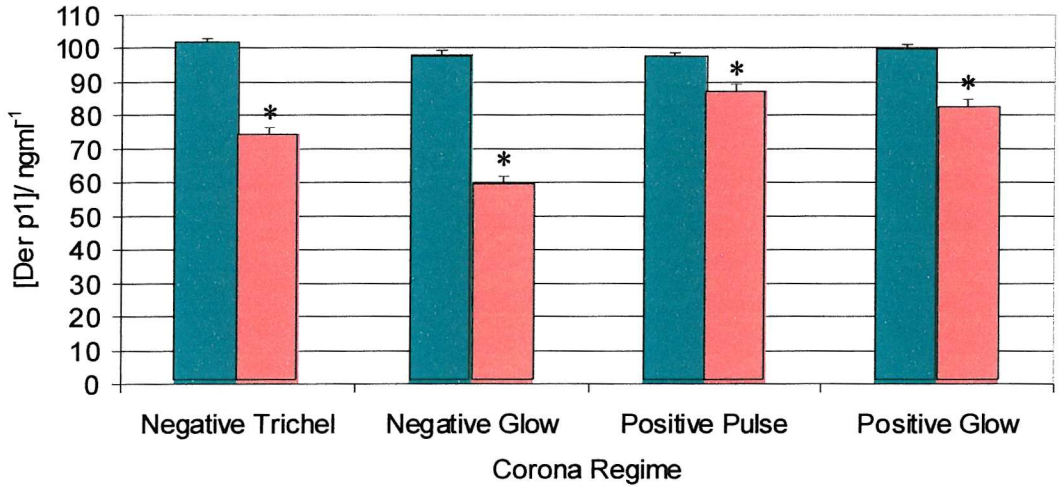


***Figure 3.48* The Der p1 concentrations in the samples exposed to corona discharge at 10%RH and their controls.**
* = Samples (■) statistically lower than their controls (■), $n = 12$, SEM shown.

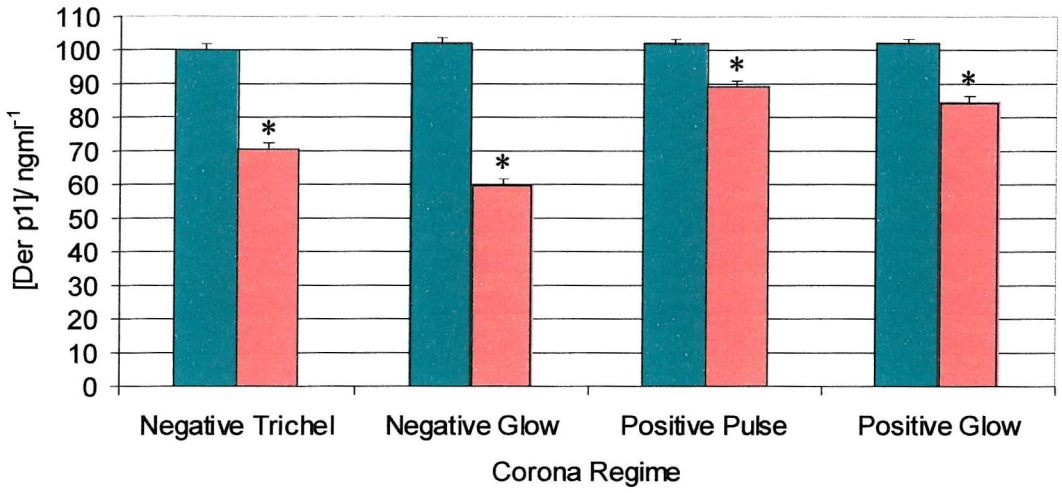


Figure 3.49 **The Der p1 concentrations in the samples exposed to corona discharge at 20%RH and their controls.**
* = Samples (■) statistically lower than their controls (■), n = 12, SEM shown.

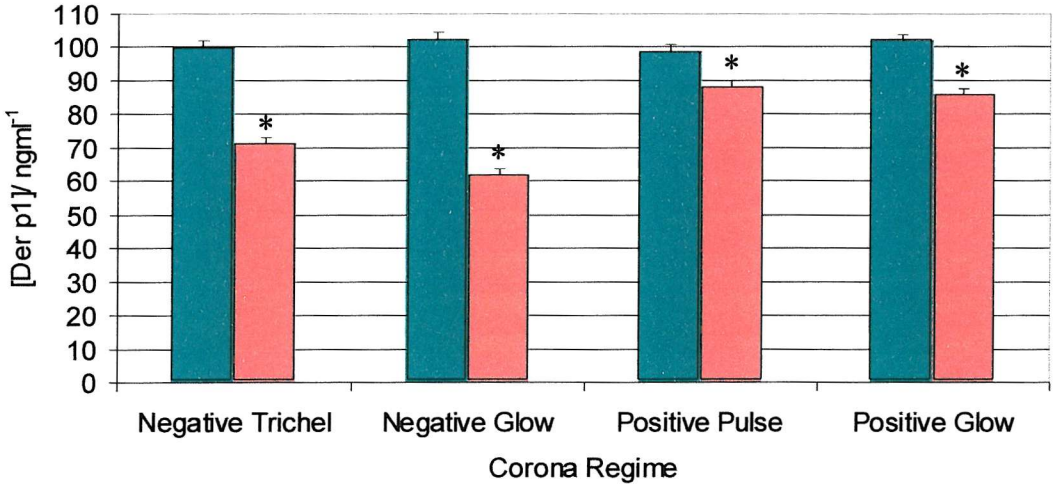


Figure 3.50 **The Der p1 concentrations in the samples exposed to corona discharge at 50%RH and their controls.**
* = Samples (■) statistically lower than their controls (■), n = 12, SEM shown.

As the initial Der p1 concentration was constant at approximately 100ngml^{-1}, percentage reduction calculations could be made. *Figure 3.51* shows the percentage reductions in Der p1 concentration achieved in the samples exposed to the different corona regimes under atmospheres of different relative humidities. As can be seen, all reductions caused by a specific regime are relatively constant over the range of RH investigated except for the negative polarity corona at 2.4%RH. The mean reduction in Der p1 concentration caused by negative Trichel corona at 10%, 20% and 50% was 28.13 ±1.56% (n=36), negative glow corona caused a mean reduction of 29.77 ±2.90% (n=36). These results are similar to those recorded at the equivalent time of 120 minutes and at 45%RH during the investigation into the effect of exposure time on Der p1 (*Section 3.11*). Positive pulse, under the entire range of relative humidities investigated, caused a mean reduction of 10.44 ±1.20% (n=48). Positive glow caused a mean reduction of 15.91±1.24% (n=48). These reductions in Der p1 concentration by positive corona are also similar to those achieved in *Section 3.11*.

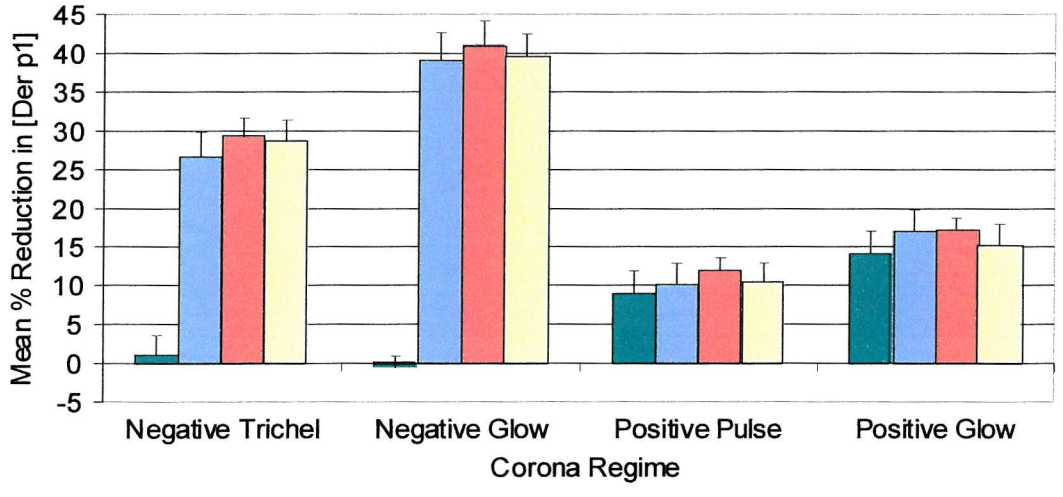


***Figure 3.51* The mean percentage reductions in the samples exposed to corona discharge at different RH levels.**
(■) = 2.4%RH, (■) = 10%RH, (■) = 20%RH, (□) = 50%RH, n = 12, SEM shown.

The only significant difference (when the reductions were compared using the Mann Whitney-U test) between the corona regimes' efficacy to reduce Der p1 concentration, at the different RH values investigated, was negative corona's ability at 2.4% and 10%RH. It would appear that for this polarity corona there is a 'cut-off point' between these two RH values i.e., a minimum level of moisture in the air is necessary for the Der p1 to be destroyed. There appears to be no such minimum value for the positive coronas, unless it exists below the lowest level of RH tested. It is this difference between negative and positive coronas that suggests that the two polarities use different reaction mechanisms to reduce the concentration of the Der p1 protein in the sample.

When a negative corona occurs in air at an RH greater than 10%, a semi-liquid layer rapidly forms on the anodic surface (the location of the Der p1 sample) (Goldman, Goldman & Sigmond, 1985). This layer contains water, NO_x^- ions from the air and other dissolved impurities. When irradiated by ions, this layer appears to behave like any other aqueous electrolyte cell i.e., the reactions that occur when a current is passed through an aqueous solution are the same for the two processes (Goldman, Goldman & Sigmond, 1985). This enables analogies to be made between corona and electrolysis. For example, in both electrolysis and as observed during corona discharge, when aluminium is used as the anode, pronounced pitting occurs (see *Section 1.8*). Under dry conditions, pitting does not occur but negative coronas cause only uniform anodic passivation (the production of a thin film of oxide that prevents any further reaction from happening) of this metal, building up surface layers charged to several hundred volts (Goldman, Goldman & Sigmond, 1985). This passivation of the aluminium foil may have also resulted in no further reaction of the corona products with the Der p1 protein.

It is also possible that the protein molecules, or the active species produced by the corona, need to be at a certain level of hydration before the negative corona products can destroy them, however, the results from the exposures to positive coronas suggest less of a dependency upon the degree of hydration for its efficacy. This might explain the differences between the negative and positive coronas' ability to destroy the Der p1 at low relative humidity.

At the low RH of 2.4%, the particular negative, active species responsible for the destruction of Der p1, observed at higher levels of humidity may not have been produced. Hydrated ions such as $O_3^-(H_2O)$, $CO_3^-(H_2O)$, $O_2^-(H_2O)_2$, $O_2^-(H_2O)_3$, $CO_4^-(H_2O)$, $NO_2^-(H_2O)$ which are produced by reactions in the interelectrode gap under ambient atmospheric conditions (Bastien, Haug & Lecuiller, 1975) may not be present at the low humidity investigated here. At the present time, no detailed study into the nature of corona products at different humidities exist, although the data presented here suggests such a difference and warrants future research into the nature of corona products responsible for allergen destruction.

3.14 THE EFFECT OF MOLECULAR OZONE ON DER P1, DER F1, DER P2 AND FEL D1

Figure 3.52 shows that no difference in allergen concentration occurred after exposure of the evaporated samples of all allergens to 50ppm ozone. However, when ozone was bubbled through the aqueous solution of each allergen a total destruction of all allergens occurred (see *Figure 3.53*). The latter method of ozone exposure is called ozonolysis and is used industrially to destroy organic matter. A number of amino acids are known to be oxidised by ozone including cysteine (Mudd, Leavitt, Ongun *et al.*, 1969). Oxidation of the cysteine residue within a protein would lead to the breaking of the disulphide bond between residues and the loss of structure and function of the protein (see *Section 3.11*). Loss of enzymatic activity has been reported after ozonolysis of ribonuclease (Mudd, Leavitt, Ongun *et al.*, 1969). This loss of activity correlated well with the oxidation of the susceptible amino acids within the protein structure.

However, the former method of ozone exposure investigated here used the protein sample in the same experimental conditions present during exposure to corona discharge as described throughout this investigation. It is possible that there is insufficient water present to react with the ozone to produce hydrogen peroxide leading to the destruction of any allergen present in the sample, although during negative corona at relative humidities above 10% the allergen sample would have a surface layer of water molecules (Goldman, Goldman & Sigmond, 1985). The results reported here are analogous to those reported by Mudd *et al.*, (1969) who found that more amino acid residues were susceptible to oxidation by ozone when in an aqueous solution than when the solvent was anhydrous (without water).

The tests described here appear to indicate that molecular ozone is not the corona product responsible for the destruction of allergen. This would mean that research directed at increasing the production of the species that are active against allergens, and decreasing the amount of deleterious ozone would be beneficial for any practical application of

corona discharge to remove allergens from the domestic environment. This is discussed further in *Chapter 5*.

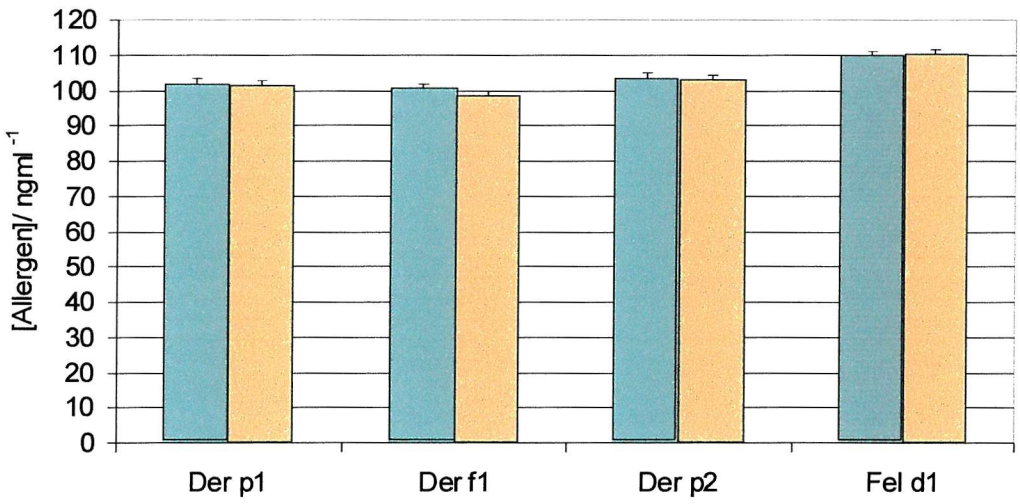


***Figure 3.52* The allergen concentrations of the evaporated allergen samples (□) exposed to ozone for 1 hour and their paired controls (□).** No difference between the controls and samples were found (n=8, SEM shown).

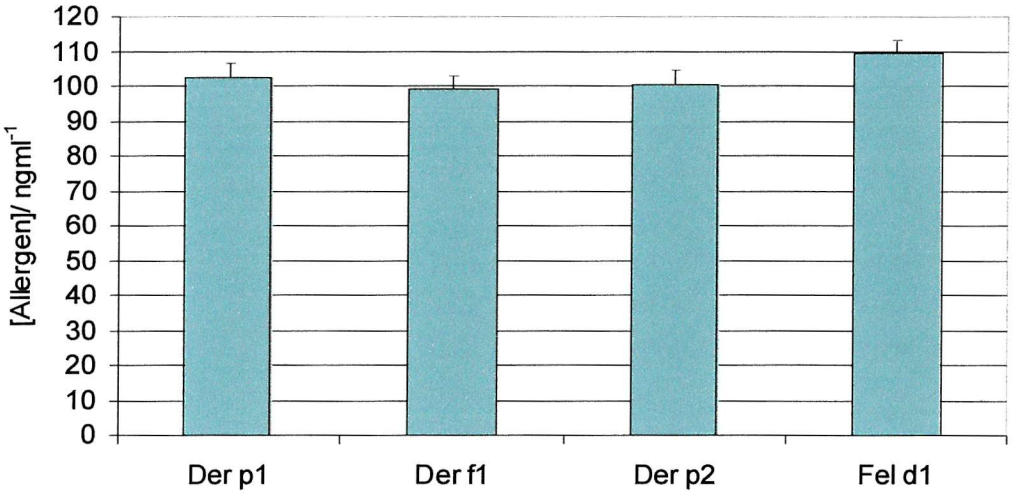


***Figure 3.53* The control concentrations of the allergen solutions.** The paired samples were subjected to ozone for 1 hour, which caused a 100% reduction in allergen concentration. (n=8, SEM shown).

3.15 THE EFFECT OF ALUMINIUM PRODUCTS ON DER P1

After exposure to the negative continuous glow corona discharge for 120 minutes a circular layer of white aluminium oxides were visible where the foil had been directly beneath the pin. *Figure 3.54* shows the Der p1 concentration of the samples exposed to these aluminium oxides and their controls. There was no statistical difference between the Der p1 samples exposed to aluminium oxides and the controls exposed to aluminium. The controls had a concentration of 100.69 ±2.09.ngml^{-1}, whereas the samples had a concentration of 99.72 ±3.49ngml^{-1}.

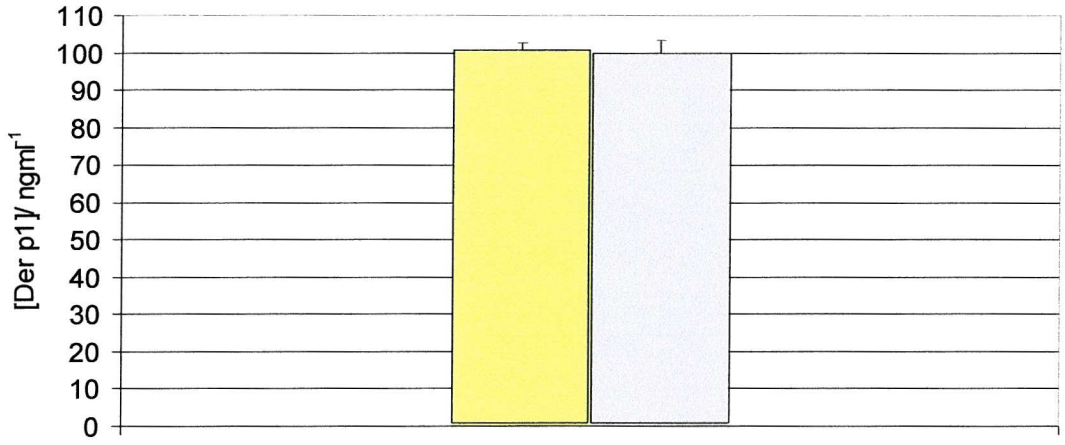


***Figure 3.54* The Der p1 concentrations in samples exposed to aluminum oxides and their controls.**
The Der p1 samples (□) were exposed to aluminium oxides produced during negative corona discharge. Controls (□) were exposed to aluminium foil (n=10, SEM shown).

This experiment showed that the aluminium oxides produced during corona discharge and present in the samples to be assayed by ELISA were not responsible for the loss in Der p1 concentration observed when samples were evaporated onto aluminium foil and exposed to corona discharge as described in previous sections of this thesis. It is more likely that the products of corona discharge itself are responsible for the destruction of

Der p1. Although this investigation focussed on the Der p1 allergen, it is likely that Der f1, Der p2 and Fel d1 would also not be affected by the aluminium products.

Another possibility was that any aluminium products present in the sample interfered with the anti-Der p1 monoclonal capture antibodies of the ELISA. Any interference with these antibodies would have led to a false negative result in the assay for the presence of Der p1. This possibility has also been ruled out by this experiment.

3.16 INVESTIGATION INTO WHETHER DER P1 CAN RENATURE AFTER EXPOSURE TO CORONA DISCHARGE

This series of experiments gives no indication that the Der p1 protein is capable of renaturing after exposure to negative corona discharge over the time periods investigated. *Figure 3.55* shows the concentration of Der p1 in the controls and samples after exposure to negative corona and stored with no buffer solution. All samples were reduced by highly statistically amounts when compared with their controls using either the paired t-test, or the Mann Whitney-U test, as appropriate ($P<0.01$).

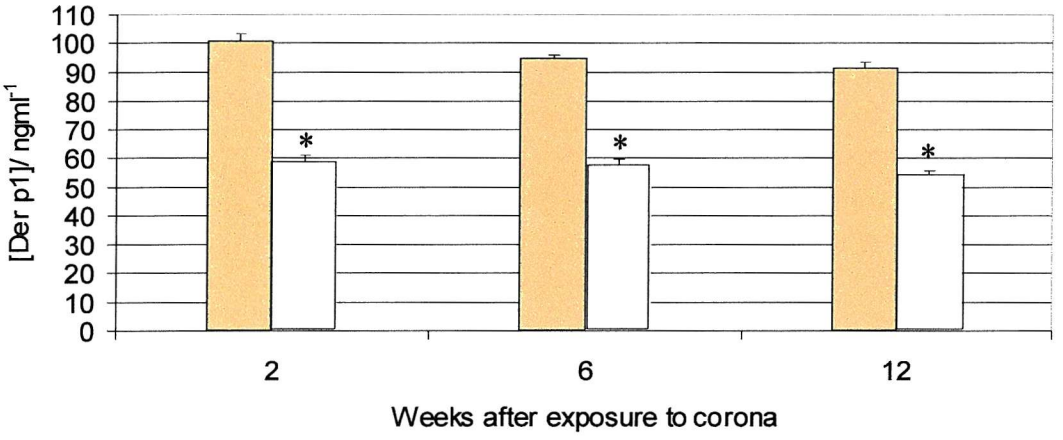


***Figure 3.55* The Der p1 concentrations in the controls and samples, stored without buffer solution for two, six and twelve weeks after exposure to negative corona discharge.**
Samples (□) were exposed to 90μA, negative continuous glow corona for 60 minutes, controls (□) were exposed to the ambient atmosphere for the equivalent period. These were then kept at 4°C. (*= statistically lower than the control, n=6, SEM shown).

Figure 3.56 shows the concentration of those samples and controls stored in buffer solution. This graph is very similar to *Figure 3.55*, and the samples in this experiment were also highly statistically lower in concentration than their paired controls ($P<0.01$). A

slight decrease in the Der p1 concentration of the controls and samples stored under both conditions can be seen over the 12 weeks, which is due to natural deterioration of the samples. No increase in the sample concentration was found in the samples stored under both conditions. This indicates that no renaturing took place over the long time period investigated.

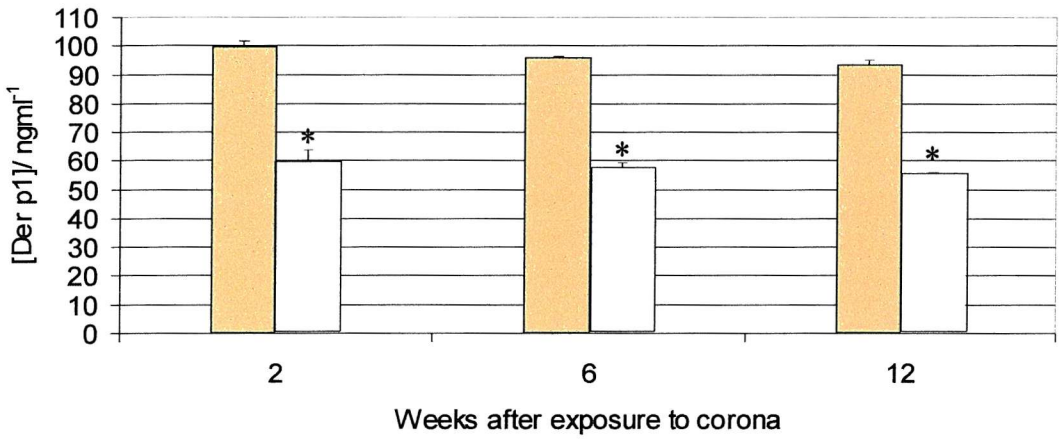


Figure 3.56 **The Der p1 concentrations in the controls and samples, stored in buffer solution for two, six and twelve weeks after exposure to negative corona discharge.**
Samples (□) were exposed to 90μA, negative continuous glow corona for 60 minutes, controls (■) were exposed to the ambient atmosphere for the equivalent period. These were then dissolved in 0.05% PBS-T and kept at 4°C. (*= statistically lower than the control, n=6, SEM shown).

Figure 3.57 clearly shows the lack of any difference between the mean percentage reductions achieved in the Der p1 concentration and the amount of time kept in storage, either dry or dissolved in buffer. Analysis by the Kruskal-Wallis test confirmed that these different data groups were not significantly different (P=0.999). The overall mean percentage reduction achieved was 40.12 ±1.07% (n=36). This corresponds well with the

result recorded in *Section 3.11* during the investigation into the effect of exposure time on Der p1.

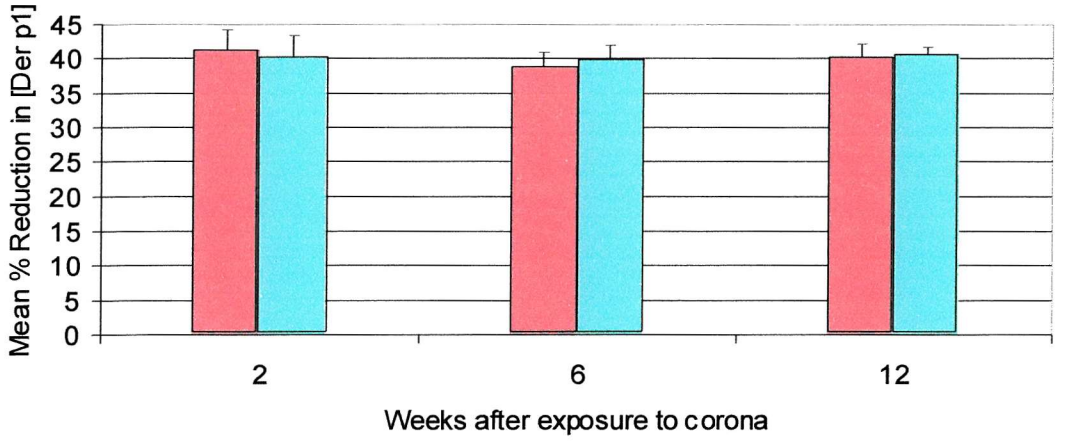


***Figure 3.57* The mean percentage reductions in the samples exposed to negative corona for sixty minutes then stored under different conditions for two, six and twelve weeks.**
(■) = Stored under dry conditions, (■) = dissolved in 0.05% PBS-T. Note that the reductions did not decrease over time and so indicate that no renaturing occurred over the time periods investigated. (n=6, SEM shown).

The lack of differences between the sample concentrations over the 2, 6 and 12 weeks post-exposure to negative corona show that no renaturing occurred whether stored with, or without, buffer of the optimum pH (pH7.35) for the protein. This gives an indication of the mechanism that the active corona product(s) use to destroy the allergen in the samples. The tertiary conformation of the protein is unlikely to have altered under the novel conditions present in the exposure to corona due to loss of hydrogen bonding or the reversible reduction of the disulphide bonds (as with a gentle increase in temperature, or decrease in pH; see *Section 1.10*). It is more probable that oxidation of the CO, or NH bond, within the peptide bonds led to degradation of the protein into its constituent amino acids, which would have been further degraded by the corona products encountering C-O, C=O, and C-OOH bonds which action by corona discharge has been shown to break (Demuth, 1984; Briggs, 1983; Mayoux, Noel *et al.*, 1983). Non-reversible oxidation of

the disulphide bonds between cysteine residues could also have played a role in destroying the allergen.

These non-reversible chemical modifications would have led to a loss of the conformational structure of the protein and so rendered it undetectable by the anti-Der p1 antibodies in the ELISA.

As mentioned in *Section 1.5.2*, some researchers have reported that Der p2 has its human IgE binding activity increased after denaturation by heat or a change in pH (Tovey, Ford & Baldo, 1989). Due to the similarities between the response of Der p2, and the other allergens, to exposure to corona discharge, and the similarities in protein structure between the allergens it is likely that this allergen would react in a similar way to the presence of corona products. Therefore, as the Der p2 protein is unlikely to be denatured but non-reversibly modified and/ or cleaved, it's IgE binding activity would not be expected to increase.

The absence of renaturing is favourable to any future application of corona discharge to reduce the allergen load of the domestic environment. Experiments into the use of an experimental, and commercially available, ionisers to reduce the allergen content of samples in chamber tests and *in situ* are described in *Chapter 4.* In these tests, any destruction of the Der p1 molecule after exposure to the ionisers would be non-reversible. The allergen load of the treated room would only rise due to natural replacement of the allergen by the house dust mites.

CHAPTER 4: LONG-RANGE DESTRUCTION OF DER P1 USING EXPERIMENTAL AND COMMERCIALLY AVAILABLE IONISERS

MATERIALS AND METHODS

As described in *Section 1.10*, ionisers have been tested for any clinical benefits they may impart to asthma sufferers. The direct action of the ions themselves, together with the filtration and precipitation of airborne dust and allergens are the properties that have been investigated thus far. The results of these tests have been contradictory and at present no conclusive clinical benefit has been demonstrated, and so their use in the domestic environment to alleviate, or prevent, the symptoms of asthma and allergies is not recommended (Colloff, Ayres, Carswell *et al.*, 1992).

This series of experiments investigated a property of ionisers that had not previously been investigated – the efficacy of corona discharge to destroy allergens. An experimental ion-wind generator and two commercially available ionisers were tested. The experimental ioniser and a commercially available ioniser that was designed for use in wardrobes as a deodorising device were tested in an electrically-grounded chamber which served as a simple model of a natural small room. The commercial ioniser, the *Ionic Closet Dry Cleaner*™ (Model: S1630, *Smarter Image Design*™, San Francisco, USA) used two wire-to-electrode configurations (electrode length: 101mm) to produce negative corona (see *Figure 4.1A*).

The experimental ioniser and a larger commercially available ioniser were also tested in an unoccupied, furnished room for their ability to reduce the Der p1 concentration of samples. The larger ioniser, the *Ionic Breeze*™ *Silent Air Purifier* (Model: S1624, *Smarter Image Design*™, San Francisco, USA) used three wire-to-electrode configurations (electrode length: 478mm) to produce negative corona (see *Figure 4.1B*).

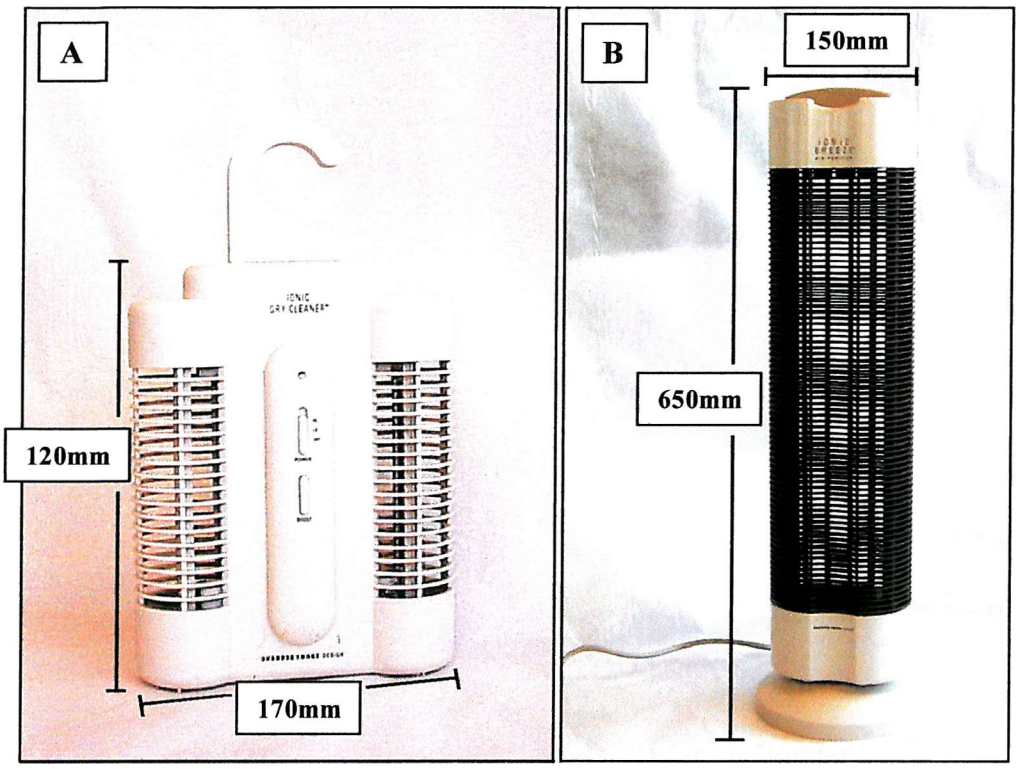


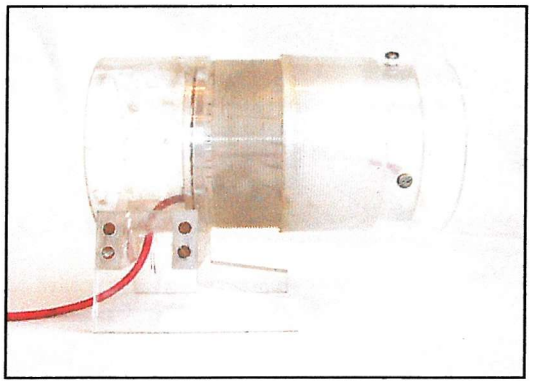
***Figure 4.1* Photographs of the commercial ionisers used in *Chapter 4*.**

A) The *Ionic Closet Dry Cleaner*™ ioniser, used in the chamber tests.

B) The *Ionic Breeze*™ *Silent Air Purifier* ioniser used in the room-scale exposure tests.

4.1 CONSTRUCTION OF A NINE-PIN EXPERIMENTAL ION WIND GENERATOR

The experimental ion wind generator was constructed by soldering nine pins to a vertical grille of 10mm^2 mesh. This was then fixed into a plastic base 30mm from a similar grille connected to earth (see *Figure 4.2*). The grille with the point electrodes could then be connected to a negative d.c., 15kV high voltage generator (Model 3807: Alpha Series III, *Brandenberg Ltd.*, Surrey, England). This nine-pin experimental ioniser was used in the experiments described in the following sections.



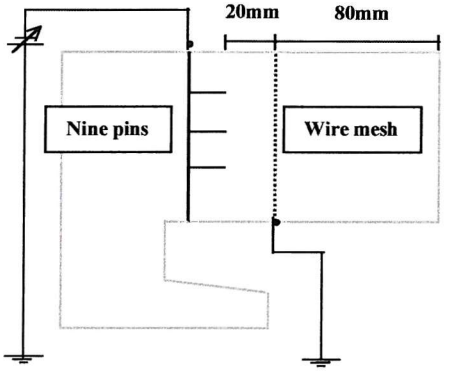


***Figure 4.2* Photograph and schematic diagram of the experimental nine-pin ion wind generator used in *Chapter 4*.**

4.2 MEASUREMENT OF THE RATE OF ION PRODUCTION AND ION WIND VELOCITY FROM THE IONISERS

See *Figure 4.3* for a diagram of the apparatus used to measure the rate of ion production. The ioniser under test was placed onto a $1m^2$ sheet of Perspex (10mm thick) and connected to its power supply. An aluminium foil-covered frame of 770 x 440 x 340mm dimensions was then placed over the ioniser to act as the ion collector and then connected to an electrometer (Model: 610C, Solid state electrometer, *Keithley Instruments*, Ohio, USA).

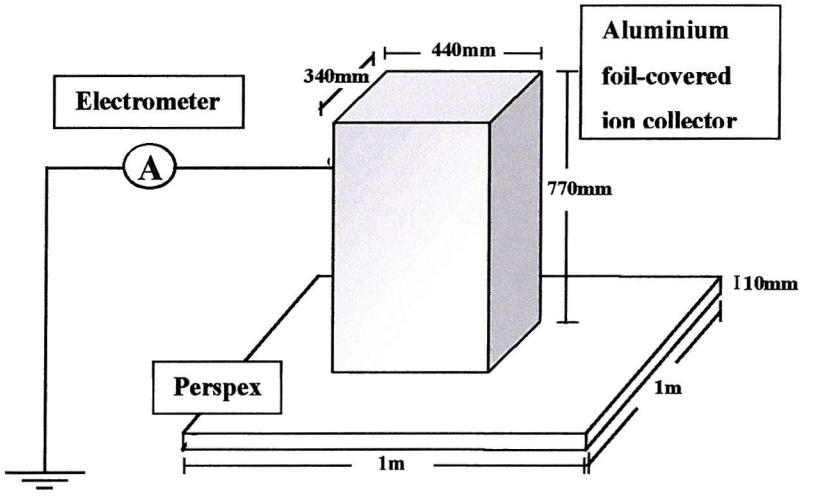


***Figure 4.3* Diagram of the apparatus used to measure the total rate of ion production from the experimental and commercial ionisers.**
This apparatus was placed inside an electrically-grounded chamber (see *Figure 4.4* for dimensions) to screen from background interference. The ioniser was placed inside the ion collector and connected to its power supply. The resultant current flow through the ion collector was measured using the electrometer.

The apparatus was all placed inside an electrically-grounded chamber to screen from background interference. The ioniser was then switched on and the resultant current flowing through the frame was measured. The rate of monovalent ion production could then be calculated using the simple formula,

$$Q = It$$

where Q was the charge collected from the ion collector in Coulombs (C), I was the current recorded from the ion collector in Amperes (A), and t was the time in seconds (s). Once the value of Q was known, the number of negative, monovalent ions produced could then be calculated by dividing this value with the charge of an electron (1.66033×10^{-27}C).

This protocol was followed to test the nine-pin experimental ion wind generator, the *Ionic Closet Dry Cleaner*™, and the larger *Ionic Breeze*™ *Silent Air Purifier*. The velocity of the ion wind was also measured for all three ionisers using an air velocity and flow meter (Model: AV2; *Airflow Developments Ltd.*, Buckinghamshire, England). Results are shown in *Section 4.6*.

4.3 CHAMBER-TESTS OF THE NINE-PIN ION WIND GENERATOR AND THE *IONIC CLOSET DRY CLEANER*™ TO DETERMINE THEIR EFFECT ON SAMPLES OF DER P1.

The chamber was made of Perspex mounted onto a metal frame. The inside surfaces of the chamber were coated with aluminium foil and tested to ensure that the entire chamber was electrically continuous and earthed (see *Figure 4.4* for a diagram of the chamber with dimensions and relative positions of the samples). A Der p1 solution was prepared according to the protocol outlined in *Section 3.1*. Samples were prepared by pippetting six, 100µl aliquots of the Der p 1 solution onto 80mm x 60mm rectangles of aluminium foil. The group of six Der p 1 samples were prepared adjacently to their paired controls. These were then all dried at 37°C.

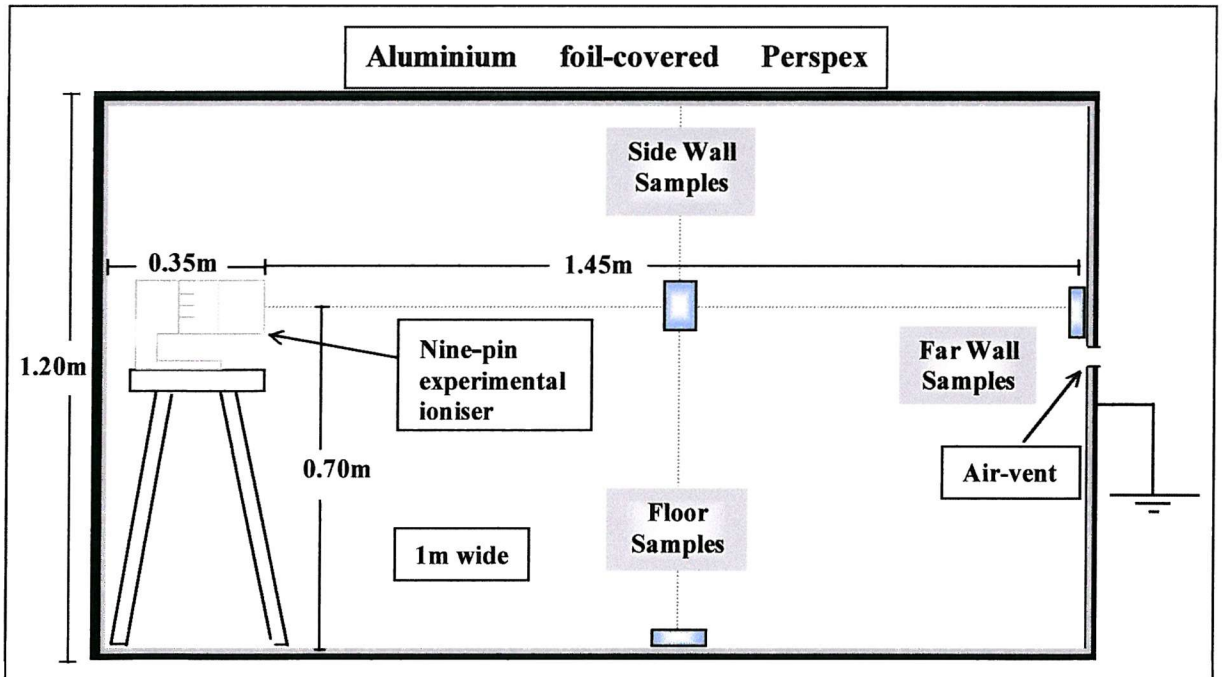


***Figure 4.4* The test chamber showing the relative positions of the ioniser and Der p1 samples.**

The nine-pin experimental ioniser is shown in position. The *Ionic Closet Dry Cleaner*™ was located in the same position facing the far wall for each test.

The ion wind generator was placed at one end of the chamber 0.70m high. The foil with the six samples was then fixed at various positions in the test chamber, as indicated in *Figure 4.4*. Six samples were placed on the far wall of the chamber, 1.45m away and directly in front of the ion wind generator; six were placed 0.70m below the generator and 0.72m away on the floor, and six were placed on the sidewall 0.50m to the left and 0.72m away from the generator.

The foil-lined chamber was then connected to ground and the ion wind generator connected to a negative applied d.c. voltage of 15kV using a high voltage generator. The Der p1 samples were exposed for one, two or three weeks. Ozone readings were taken once a week through a 20mm x 100mm air vent in the far wall of the chamber using short-term ozone detecting tubes (*Drager, Sicherheitstechnik GmbH*, Germany). Controls were kept in an identical chamber for the equivalent duration.

After exposure, the samples and controls were removed and added to 1% BSA-PBS-T in a 1:6 dilution in preparation for Der p1 concentration analysis by a two-site monoclonal antibody ELISA (*INDOOR Biotechnologies Ltd.*, Cardiff, UK).

This protocol was repeated using the commercially available small *Ionic Closet Dry Cleaner*™ (Model: S1630, *Smarter Image Design*™, San Francisco, USA) designed for use in wardrobes. The *Ionic Closet Dry Cleaner*™ was placed in the same position as the nine-pin ion wind generator facing the far wall and connected to a d.c., 12V mains transformer for the exposure periods. The results are shown in *Section 4.7*.

4.4 ROOM-SCALE EXPOSURE OF DER P1 SAMPLES TO THE NINE-PIN ION GENERATOR AND THE *IONIC BREEZE™ SILENT AIR PURIFIER*

Samples of evaporated Der p1 solution were prepared according to the protocol outlined in *Section 3.1.* Twelve groups of six evaporated Der p1 samples were placed in an unoccupied, furnished, office room measuring 2.9 x 6.0 x 2.9m (see *Figure 4.5* for a map of the samples in the room and the positions of the furniture). The majority of samples were fixed horizontally and at the same height as the nine-pin ion wind generator that was placed on a table at one end of the room. The samples were positioned in radial sectors equidistant from the ioniser at 0.3, 1.2, 2.4 and 4m for Sectors 1, 2, 3 and 4 respectively. Samples 2.2 and 4.2 were placed on the floor 1.05m below the centre of the ion wind generator and Samples 3.1 and 3.3 were fixed vertically against the walls.

The windows and door of the office were closed and the ion wind generator connected to a negative d.c., 15kV power supply for one, two or three weeks. Sixteen control samples were kept in identical conditions without the ion wind generator present. The door was briefly opened every two days to measure the ozone concentration in the room. At the completion of exposure, the samples were removed and prepared for ELISA. Results are shown in *Section 4.8.1.*

The above protocol was also followed using the commercially available *Ionic Breeze™ Silent Air Purifier* (Model: S1624, *Smarter Image Design™*, San Francisco, USA), which was designed for use in rooms. The *Ionic Breeze™* ioniser was placed in the same position as the smaller nine-pin ion wind generator and connected to a 110V mains transformer for one, two or three weeks. Der p1 samples were placed in identical positions to those used in the ion wind generator exposures. This was at a height of 150mm from the bottom of the ioniser. The control samples were kept under identical conditions without the ioniser present. The door was briefly opened every two days to measure the ozone concentration and to clean the planar electrodes of the ioniser with a dry cloth in accordance with the manufacturer's instructions. After the duration of

exposure the samples and controls were prepared for ELISA. Results are shown in *Section 4.8.2.*

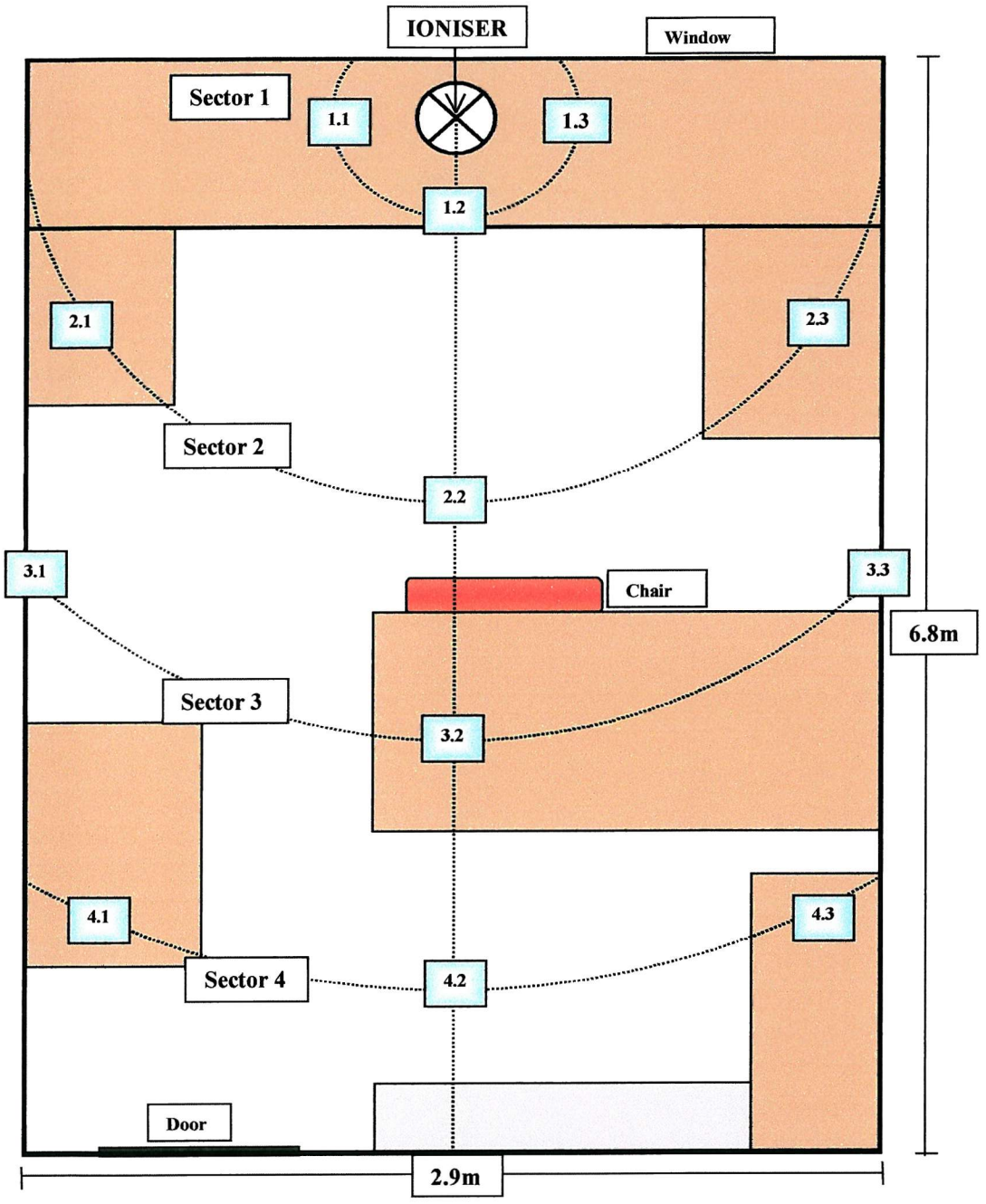


***Figure 4.5* Map of sample positions in the unoccupied office room for *in situ* tests of the nine-pin ion wind generator and the *Ionic Breeze*™ *Silent Air Purifier*.**

Three sets of six samples were arranged in radial sectors equidistant from the ioniser (Sector 1 = 0.30m, Sector 2 = 1.20m, Sector 3 = 2.40m, Sector 4 = 4.00m). The tables were of approximately the same height (0.90m), and the chair, in line with the ioniser, was 0.1m taller. Samples 2.2 & 4.2 were placed on the floor 1.05m below the level of the ioniser, and Samples 3.1 & 3.3 were fixed vertically to the walls.

4.5 MEASUREMENT OF THE EXTENT OF ACTIVE CORONA PRODUCT PENETRATION

The majority of the allergenic load within the domestic environment is found in dust reservoirs *inside* mattresses, soft furnishings, pillows etc., where conditions are favourable for mite growth (Colloff, 1998). To be effective in reducing the amount of allergen in the home, corona products from ionisers would therefore have to penetrate into these reservoirs. Three different fabrics, including upholstery fabric, and two different types of foam, of varying thickness, were tested in order to determine the depth to which the active corona product(s) responsible for allergen destruction could penetrate.

Samples of evaporated Der p1 solution (prepared according to the protocol outlined in *Section 3.1*) were prepared by pippetting twelve, 100µl aliquots of the Der p 1 solution onto 80mm x 60mm rectangles of aluminium foil. The group of twelve Der p 1 samples was prepared adjacently to their paired controls. These were then all dried at 37°C. This piece of foil was then fixed onto the floor of a shallow plastic box with dimensions of 60mm x 100mm x10mm (see *Figure 4.6*). The inside floor of the box was coated with aluminium foil and connected to earth via a wire inserted into the side of the box. An electrometer (Model 610C, Solid state electrometer, *Keithley Instruments*, Ohio, USA) was then connected in series with the ground line in order to measure the current flowing through the foil.

The majority of the lid of this box was removed so that only a 10mm top edge remained. The fabric could then be fixed into place 10mm above the samples by placing the edge of this lid over the fabric and ensuring the fabric was taut.

This fabric-covered sample box holding the Der p1 samples was then placed beneath the nine-pin experimental ion wind generator (constructed according to *Section 4.1*) used in the investigation into long-range destruction of Der p1 tests. The ioniser was clamped into a vertical position with the aperture facing the samples 100mm below (90mm above

the sample box. The pins were then connected to negative d.c., 15kV using a high voltage generator for 48 hours.

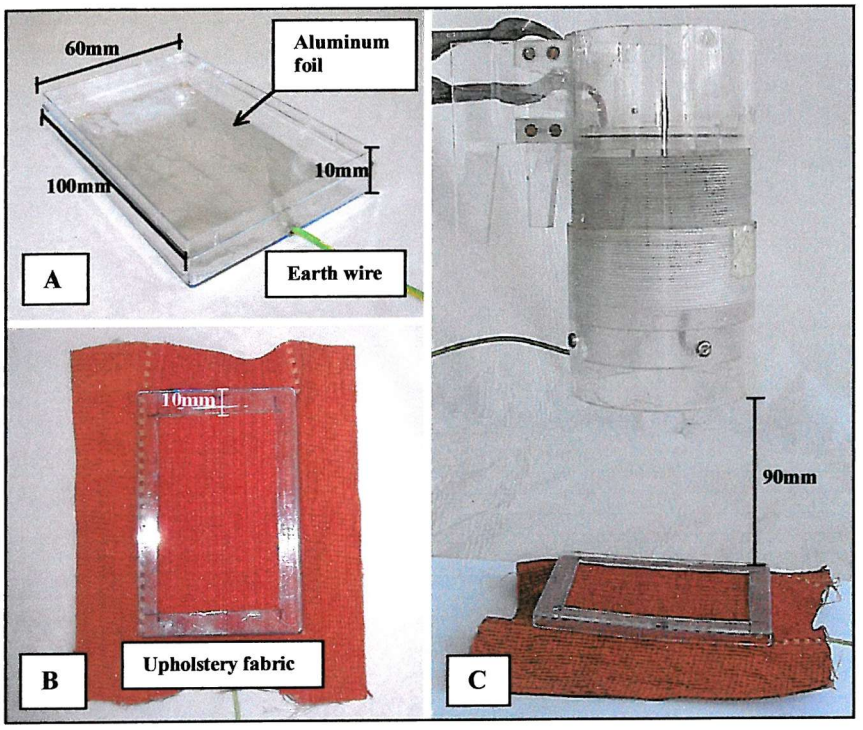


***Figure 4.6* Photographs of the apparatus used to determine the extent of active corona product penetration.**
A) The shallow plastic sample box used to hold the evaporated Der p1 samples. The sheet of foil with the samples was fixed to the aluminium foil-covered base, which was connected to earth. B) Plan view of the sample box covered in the fabric to be tested. First the fabric was placed over the box holding the samples, then the lid was placed over the fabric onto the box and the fabric pulled taut. C) Side view of the nine-pin experimental ioniser clamped above the fabric-covered Der p1 samples.

Paired, negative controls were prepared by exposing Der p1 samples to the ambient atmosphere for the equivalent period. After exposure, the samples and controls were cut

from the foil, placed in eppendorfs and prepared for ELISA to determine the Der p1 concentration. This protocol was repeated using three different fabrics 10mm above the Der p1 samples: cotton, polyester and upholstery fabric. These fabrics, measured using vernier callipers, had different thicknesses: 0.25mm, 0.20mm and 1.05mm for the cotton, polyester and upholstery fabrics respectively. Positive controls were prepared by exposing six Der p1 samples to the ioniser for 48hrs with no fabric covering the sample box. These positive controls also had their paired negative controls in order to determine the percentage reduction in Der p1 concentration caused by the ioniser alone.

In order to determine the extent to which the active corona product(s) could penetrate into soft furnishings, two different types of foam, of varying thickness were cut and fixed to the lid of the sample box in turn. The samples were connected to earth and the foam was fixed into position using duct tape to seal the edges to the plastic lid (see *Figure 4.7*). The samples were then exposed for 48 hours to the negative corona. This protocol was repeated using closed cell, expanded polypropylene foam (*Fabric and Foam*, Truro, Cornwall, England) and open cell, reticulated polyester foam (65 pores per inch; Foamex Corp., Eddystone, Pennsylvania, USA) with thicknesses of 5mm, 10mm, 25mm and 50mm. The results of these tests are shown in *Section 4.9.*

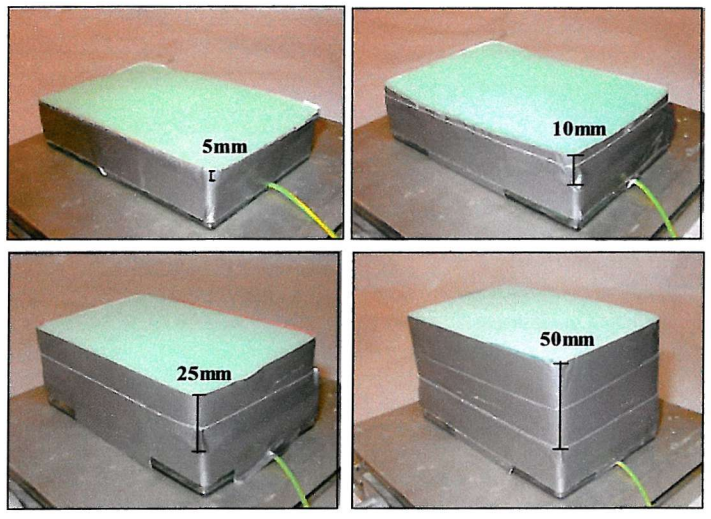


***Figure 4.7* Photographs of the different sizes of open cell, reticulated polyester foam fixed to the sample box.**

RESULTS AND DISCUSSION

4.6 THE RATE OF ION PRODUCTION FROM THE IONISERS AND THE VELOCITY OF THE ION WIND

Each ioniser had a different rate of ion production. The experimental, nine-pin ion wind generator produced 2.497×10^{13} ions per second, leading to an ion wind with a velocity of $0.23 ms^{-1}$ immediately in front of the ioniser. The commercially available *Ionic Closet Dry Cleaner*™, however, produced 2.185×10^{14} ions per second but resulted in a negligible ($<0.00 ms^{-1}$) ion wind velocity as it left the ioniser in all directions. The larger, *Ionic Breeze*™ *Silent Air Purifier* ioniser had the highest rate of ion production at 1.904×10^{15} ions per second. The velocity of the ion wind differed depending upon which side of the ioniser it was measured: from the front it was $0.69 ms^{-1}$, from the back it was $0.29 ms^{-1}$ and from the side the velocity was negligible.

4.7 CHAMBER-TESTS OF THE EXPERIMENTAL NINE-PIN ION WIND GENERATOR AND THE *IONIC CLOSET DRY CLEANER*™ TO DETERMINE THEIR EFFECT ON SAMPLES OF DER P1.

4.7.1 EXPOSURE OF DER P1 SAMPLES IN THE CHAMBER TO THE NINE-PIN ION WIND GENERATOR

The results of these chamber tests show that significant reductions in the Der p1 concentration of samples were achieved using the nine-pin ion wind generator. These reductions increased over the exposure times investigated. *Figures 4.8, 4.9 & 4.10* show the Der p1 concentrations of the samples exposed to the ion wind generator, and their controls, for one, two or three weeks. All of the samples, except those located on the floor and side wall and exposed for one week, were statistically lower in Der p1 concentration than their paired controls when compared using the paired t-test for means, or the Mann Whitney-U test where appropriate ($P<0.05$). The concentrations of the controls were relatively constant over the three weeks with a mean concentration of 227.22 ±3.89ngml^{-1} (n=54).

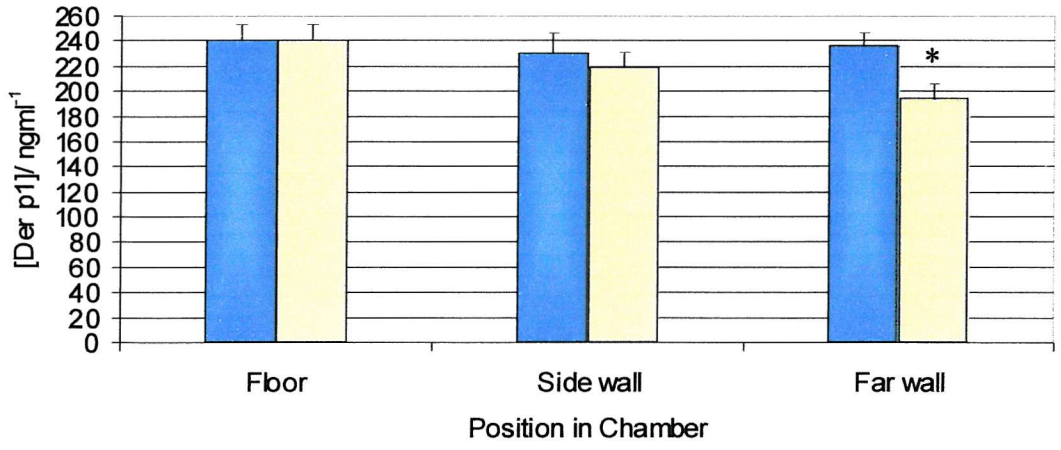


***Figure 4.8* The Der p1 concentrations of the controls and samples after 1 week exposure to the experimental ion wind generator in the chamber.** Samples (□) were exposed to the experimental ion wind generator in the chamber for 1 week. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

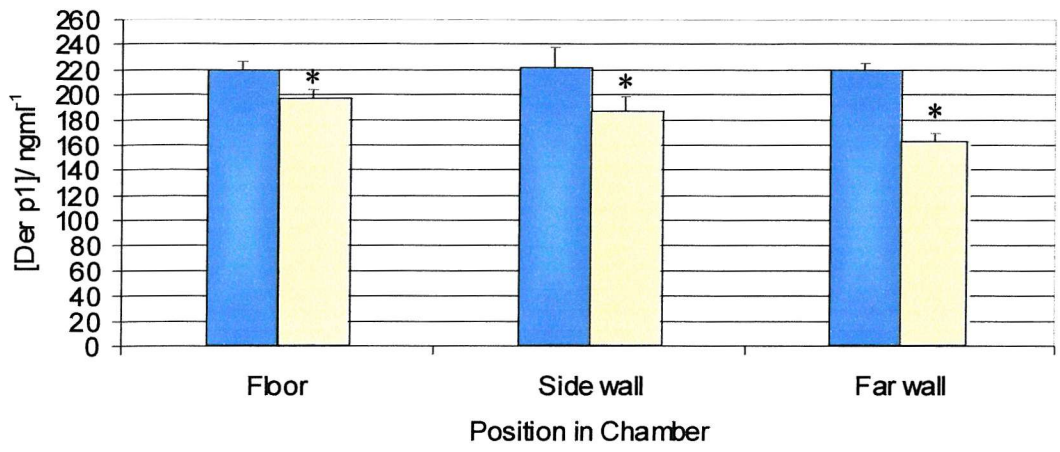


Figure 4.9 **The Der p1 concentrations of the controls and samples after 2 weeks exposure to the experimental ion wind generator in the chamber.** Samples (□) were exposed to the experimental ion wind generator in the chamber for 2 weeks. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

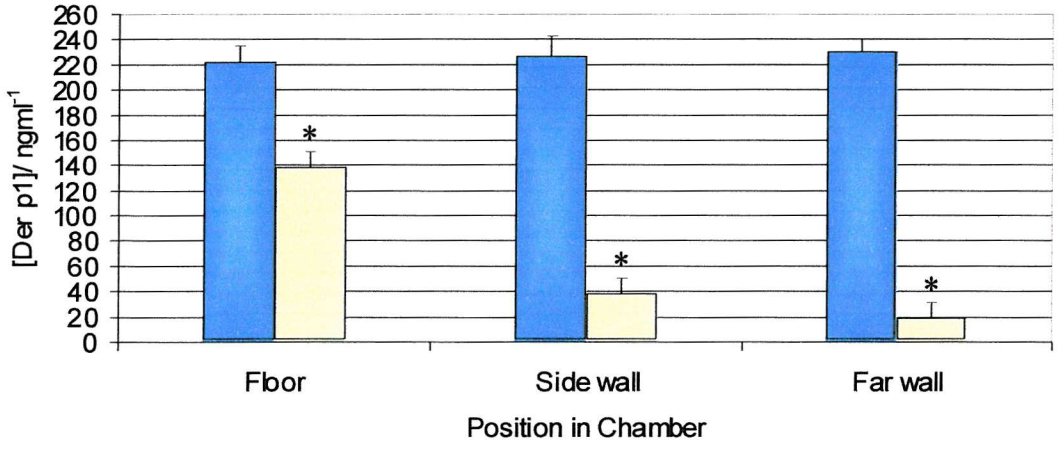


Figure 4.10 **The Der p1 concentrations of the controls and samples after 3 weeks exposure to the experimental ion wind generator in the chamber.** Samples (□) were exposed to the experimental ion wind generator in the chamber for 3 weeks. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

Figure 4.11 shows the mean percentage reductions in the Der p1 concentration of the samples. Statistically significant strong, positive correlations between the percentage reduction in Der p1 and the period of exposure were observed for all sample positions in the chamber ($P<0.01$). The greatest reduction was seen in the samples directly opposite the ion wind generator on the far wall. The percentage reductions rose from 17.70 ±4.30% after one week to 25.84 ±2.85% after two weeks and 92.12 ±2.07% after three weeks (Spearman's rho=0.826). The second greatest reductions were seen in the samples situated on the sidewall, which rose from 3.15 ±6.83% to 14.14 ±4.93% and finally 82.79 ±2.71% after three weeks (rho=0.787). The samples on the floor showed the lowest concentration reduction, with no mean percentage reductions after one week and only 10.10 ±2.01% and 37.29 ±3.73% reductions after two and three weeks respectively (rho=0.787). The ozone concentration inside the chamber remained at a constant 2.40ppm throughout the experiment.

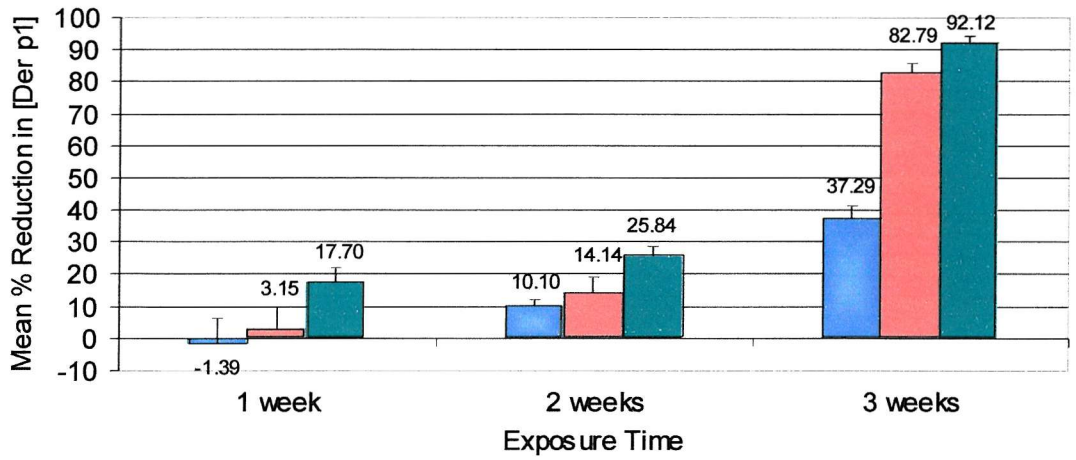


***Figure 4.11* Mean percentage reductions in the samples exposed to the experimental ion wind generator for 1, 2 or 3 weeks at different positions in the chamber**
(■) = Samples on the floor of the chamber, (■) = samples on the side wall, (■) = samples on the far wall of the chamber opposite the ioniser. (n=6, SEM shown).

4.7.2 EXPOSURE OF DER P1 SAMPLES IN THE CHAMBER TO THE *IONIC CLOSET DRY CLEANER*™

This series of chamber tests has shown that the Der p1 concentration of the samples was significantly reduced after exposure to the *Ionic Closet Dry Cleaner*™. *Figures 4.12, 4.13 & 4.14* show the Der p1 concentrations of the samples exposed to the commercially available ioniser, and their controls, for one, two or three weeks. All of the samples were statistically lower in Der p1 concentration than their paired controls when compared using the paired t-test for means ($P<0.05$). The concentrations of the controls were relatively constant over the three weeks with a mean concentration of 226.94 ±2.23ngml^{-1} (n=54).

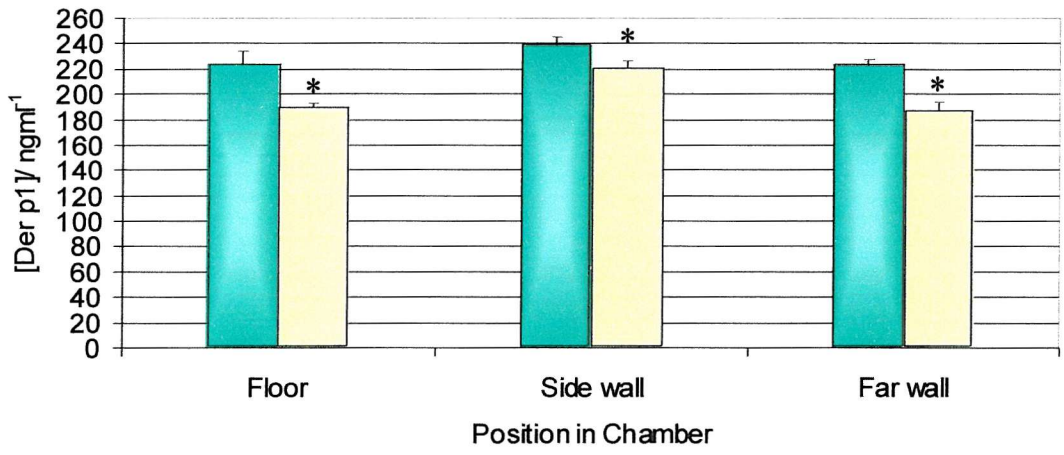


***Figure 4.12* The Der p1 concentrations of the controls and samples after 1 week exposure to the *Ionic Closet Dry Cleaner*™ in the chamber.**
Samples (□) were exposed to the *Ionic Closet Dry Cleaner*™ in the chamber for 1 week. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

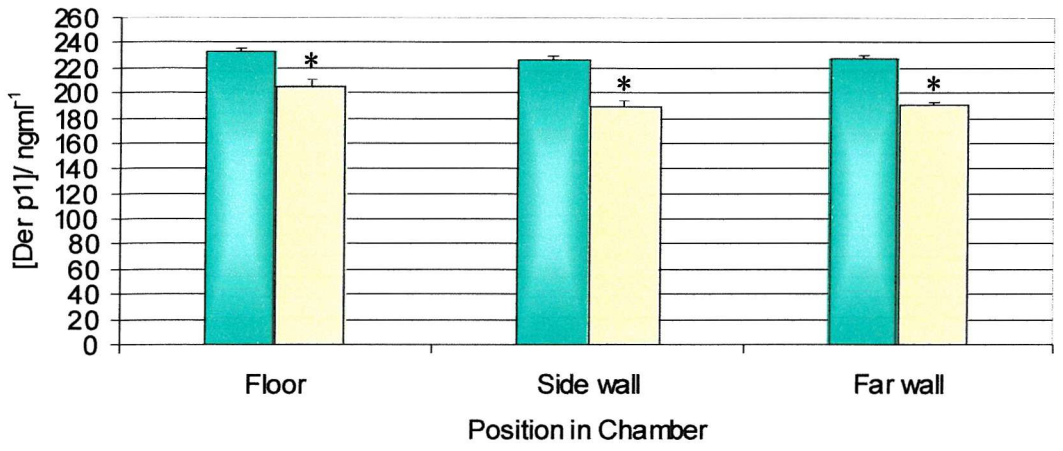


Figure 4.13 **The Der p1 concentrations of the controls and samples after 2 weeks exposure to the *Ionic Closet Dry Cleaner*™ in the chamber.**
Samples (□) were exposed to the *Ionic Closet Dry Cleaner*™ in the chamber for 2 weeks. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

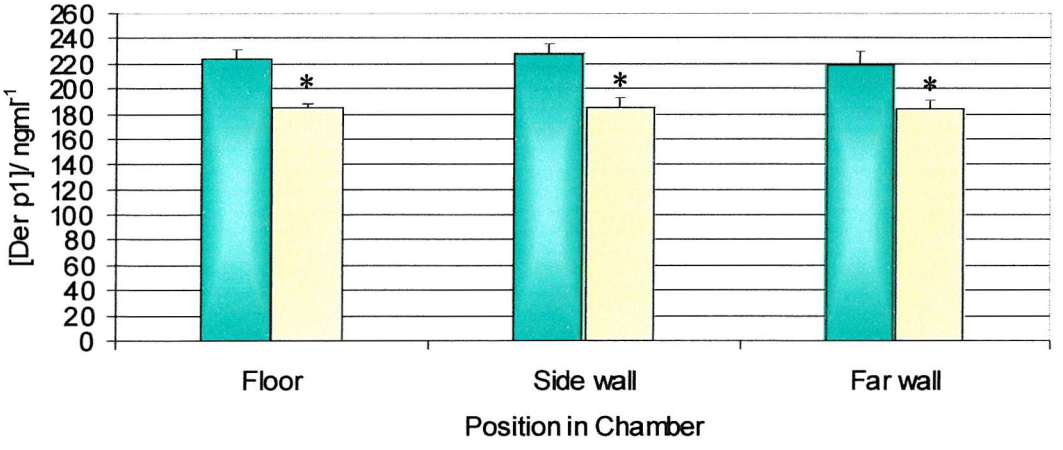


Figure 4.14 **The Der p1 concentrations of the controls and samples after 3 weeks exposure to the *Ionic Closet Dry Cleaner*™ in the chamber.**
Samples (□) were exposed to the *Ionic Closet Dry Cleaner*™ in the chamber for 3 weeks. Controls (■) were exposed to the ambient atmosphere for the equivalent period (*= statistically lower than the control, n=6, SEM shown).

Figure 4.15 shows the mean percentage reductions in the Der p1 concentration of the samples. Unlike the reductions observed with the experimental ioniser, the values of mean percentage reduction in Der p1 concentration of most samples were all relatively constant irrespective of the length of exposure. Only the samples placed on the sidewall of the chamber had successive reductions in concentration. The sidewall sample concentration reductions ranged from 7.47 ±2.67% reduction after one week, 16.65 ±1.56% after two weeks and 18.80 ±1.93% after three weeks (rho=0.669, $P<0.01$). The values for the reduction in concentration of samples in the other positions and time exposures ranged from 11.64 ±2.49% to 16.74 ±2.36% and showed no correlation between length of exposure and percentage reduction achieved. The ozone concentration inside the booth remained a constant 0.05ppm.

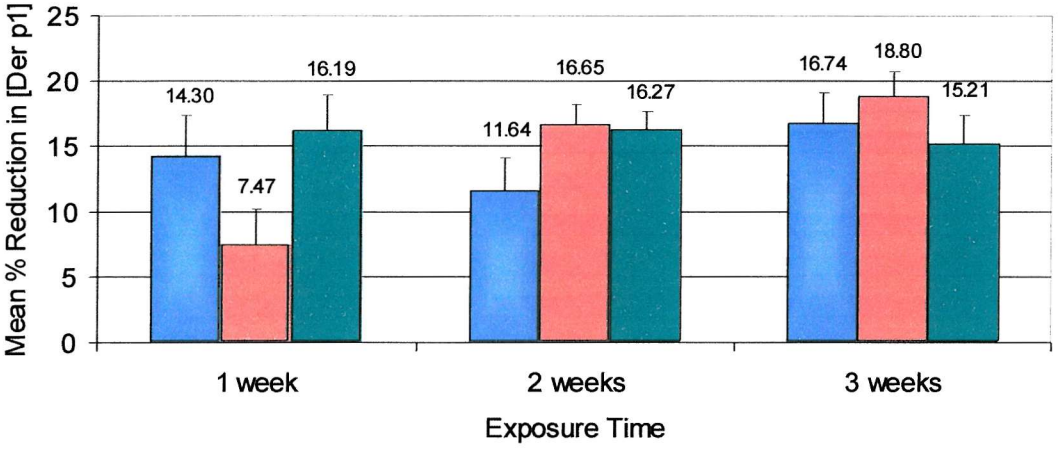


***Figure 4.15* Mean percentage reductions in the samples exposed to the *Ionic Closet Dry Cleaner*™ for 1, 2 or 3 weeks at different positions in the chamber**
(■) = Samples on the floor of the chamber, (■) = samples on the side wall, (■) = samples on the far wall of the chamber opposite the ioniser. (n=6, SEM shown).

The reductions observed in this chamber test were very different from those observed with the nine-pin ion wind generator. In the latter test, a clear relationship with both time of exposure and position of sample could be seen; the samples continued to decrease in

concentration after each week of exposure, and the samples on the far wall in front of the ion generator were reduced the most. A possible explanation for this resides in the fact that the *Ionic Closet Dry Cleaner*™ released the ionised air in a diffuse manner in all directions with a negligible velocity, whereas the nine-pin ion generator carried its corona products on the ion wind at $0.23ms^{-1}$ directly onto the samples on the far wall. Unlike the release of the ion wind from the *Ionic Closet Dry Cleaner*™ ioniser, there appears to be little diffusion of the active corona products, especially to the floor of the chamber. As the chamber is connected to ground, the far wall could capture the active species allowing little lateral diffusion to destroy Der p1 in the surrounding samples. This would suggest that the active species in the corona products are ionic, although it is also possible that the electrically neutral, but chemically reactive, radicals could be neutralised after contact with the far wall.

Although the *Ionic Closet Dry Cleaner*™ caused significant reductions in allergen concentration in each of the time exposures, the reductions in all positions were relatively constant. This was also probably due to the release of the ion wind in all directions. This method of ion release would allow much more diffusion of the active corona product and so destroy allergen in all the sample positions. It is not clear however why the reductions observed were also constant for the different exposure times; the concentration of Der p1 would be expected to decrease with an increase in exposure time.

Although the commercial ioniser had a faster rate of ion production (See *Section 3.5.1*), it only destroyed significantly more Der p1 in the samples fixed to the floor after one week of exposure (when the percentage reductions caused by the two ionisers at each sample position within each time exposure were compared using the Mann Whitney-U test, $P<0.05$). The reductions caused by the commercial ioniser in the samples positioned on the side wall and far wall of the chamber after one week, and the samples on the floor and side wall after two weeks exposure were not significantly different from those caused by the experimental ioniser ($P>0.05$). Der p1 samples on the far wall after two weeks, and samples in all positions after three weeks were reduced to a greater extent by the experimental ioniser ($P<0.01$).

Previous investigations into the effect of corona discharge on Der p1 used samples with an approximate initial concentration of 100ngml^{-1}. In the chamber tests, the initial concentration was approximately 225ngml^{-1}. This difference was due to the heterogeneous concentration of the HDM culture used to make the stock solution (see *Section 2.4*). The results from the investigation into the effect of initial allergen concentration (see *Section 2.5.1*) indicate that the percentage reductions obtained in allergen concentration of a sample refer to a specific number of allergen molecules destroyed per exposure to corona discharge in a pin-to-plane electrode arrangement. This is also likely to be the case in these long-range experiments into the effect of corona discharge on the Der p1 allergen. It is also likely that the other allergens (Der f1, Der p2 and Fel d1), tested in the pin-to-plane corona set-up, will be susceptible to damage by the corona products produced by ionisers.

These chamber tests, modelling a small room, have shown that the active product(s) of negative corona discharge can destroy the Der p1 allergen at a distance of 1.45m. The results of tests with the experimental ioniser and a larger commercially available ioniser in a furnished room are shown in the following section. In these tests the reductions in Der p1 concentration of samples up to 4m away from the ioniser are described.

4.8 ROOM-SCALE EXPOSURE OF DER P1 SAMPLES TO THE NINE-PIN ION WIND GENERATOR AND THE *IONIC BREEZE*™ *SILENT AIR PURIFIER*

4.8.1 ROOM-SCALE EXPOSURE OF SAMPLES TO THE NINE-PIN ION WIND GENERATOR

The results of these tests show that highly significant reductions in the Der p1 concentration of samples were recorded after exposure to the experimental ion wind generator for one, two or three weeks in the unoccupied, furnished office room (see *Figure 4.5* for a map of the samples in the room). *Figures 4.16, 4.17 & 4.18* show the Der p1 concentrations of the controls and samples in their various positions in the furnished office room after one, two and three weeks exposure respectively. The control concentrations were relatively constant with mean concentrations of 227.654 ±6.96ngml^{-1}, 225.268 ±4.24ngml^{-1} and 228.335 ±2.94ngml^{-1} for the one, two and three week exposures respectively. All samples were statistically lower than their controls when compared using the t-test or the Mann-Whitney-U test where appropriate (P<0.01). The ozone concentration in the room, measured at a distance of 6m from the ion wind generator, was a constant 0.05ppm throughout the one, two and three week exposures.

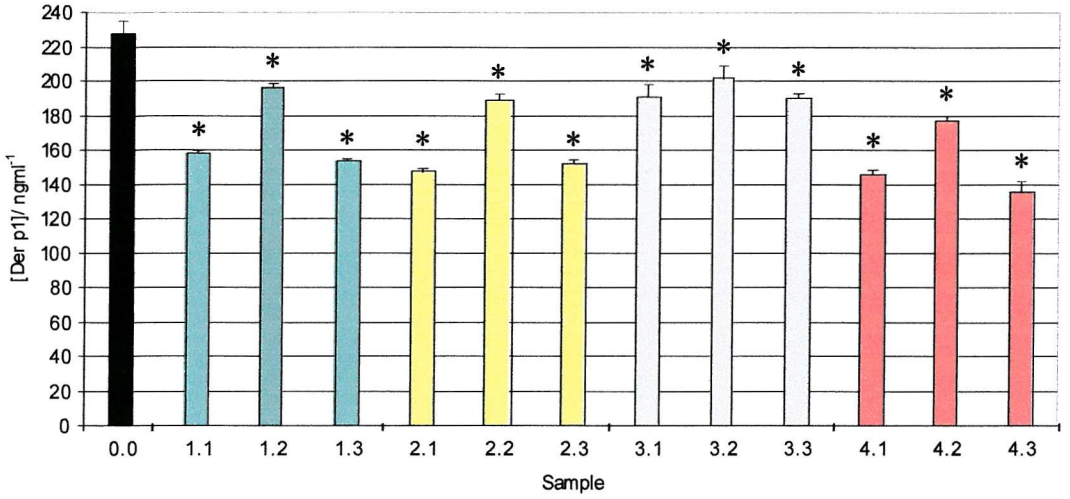


Figure 4.16 **The Der p1 concentrations of the controls and samples in various positions around the unoccupied, furnished room after 1 week exposure to the experimental ion wind generator.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5* . Controls (0.0) were exposed to the ambient atmosphere for the equivalent period. * = Statistically lower than the control. (Controls: n=16; Samples: n= 6; SEM shown).

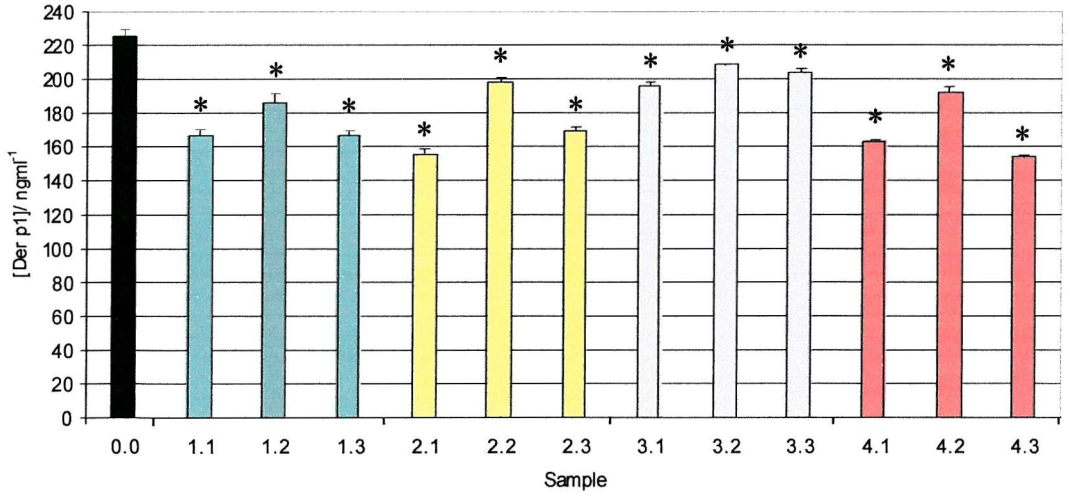


Figure 4.17 **The Der p1 concentrations of the controls and samples in various positions around the unoccupied, furnished room after 2 weeks exposure to the experimental ion wind generator.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5.* Controls (0.0) were exposed to the ambient atmosphere for the equivalent period. * = Statistically lower than the control. (Controls: n=16; Samples: n= 6; SEM shown).

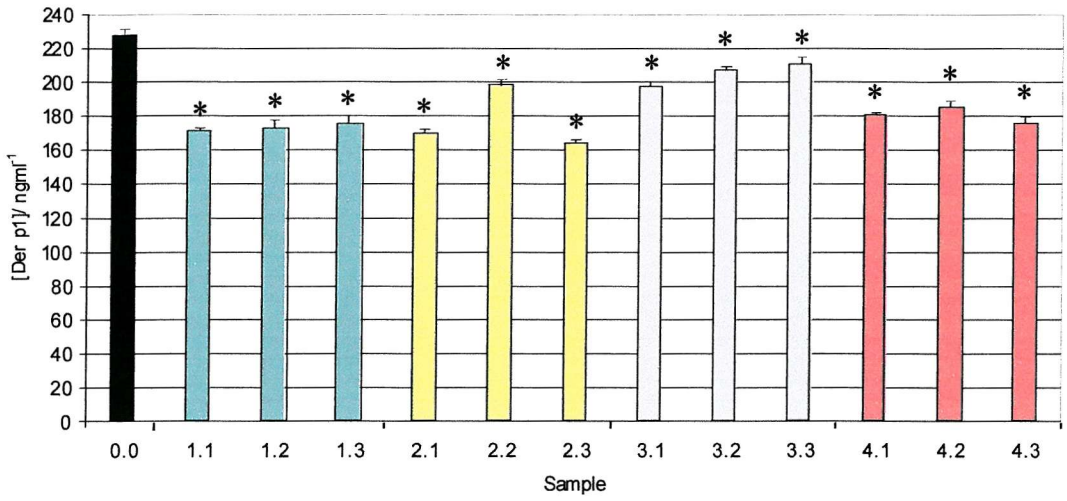


***Figure 4.18* The Der p1 concentrations of the controls and samples in various positions around the unoccupied, furnished room after 3 weeks exposure to the experimental ion wind generator.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5.* Controls (0.0) were exposed to the ambient atmosphere for the equivalent period. * = Statistically lower than the control. (Controls: n=16; Samples: n= 6; SEM shown).

Figures 4.19, 4.20 & 4.21 show the mean percentage reductions in Der p1 concentration over the three exposure periods. After a one week exposure to the nine-pin ion wind generator, reductions in the Der p1 concentration were observed that ranged from 11.49 ±3.18% at Sample 3.2 (2.4m away from the ioniser on the desk) to 40.63 ±2.83% at sample 4.3 (4m away on the table in the far corner). There appears to be no decrease in the efficacy of the corona product(s) to destroy Der p1 as distance from the ion wind generator is increased.

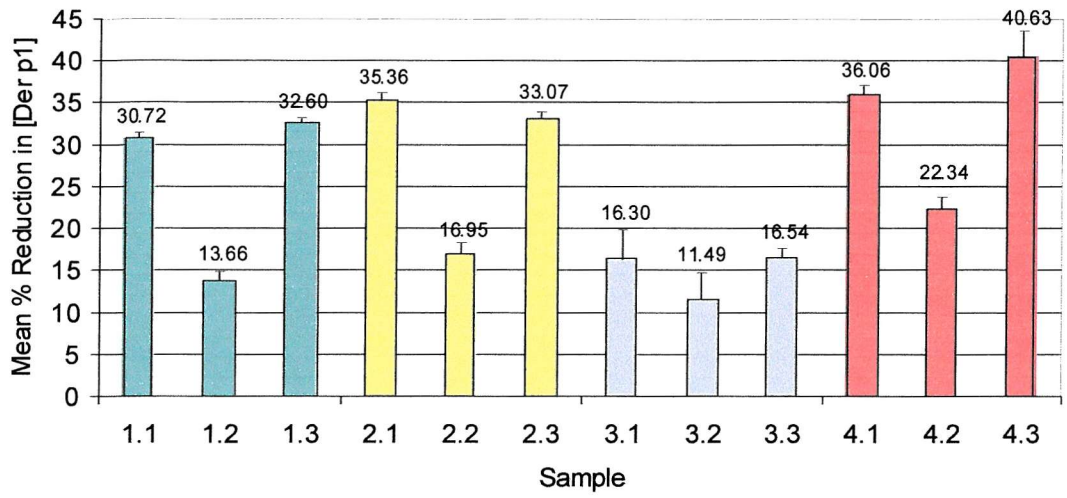


Figure 4.19 **The mean percentage reduction in Der p1 concentration of samples in various positions around the unoccupied, furnished room after 1 week exposure to the experimental ion wind generator.**
The sample numbers refer to the position in each sector as shown in *Figure4.5*. All values are statistically significant. (n= 6; SEM shown).

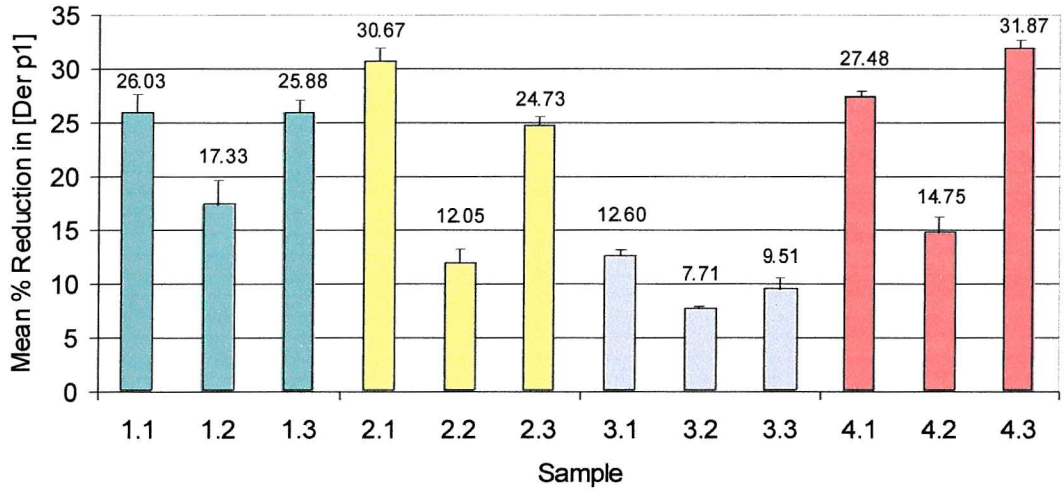


Figure 4.20 **The mean percentage reduction in Der p1 concentration of samples in various positions around the unoccupied, furnished room after 2 weeks exposure to the experimental ion wind generator.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5*. All values are statistically significant. (n= 6; SEM shown).

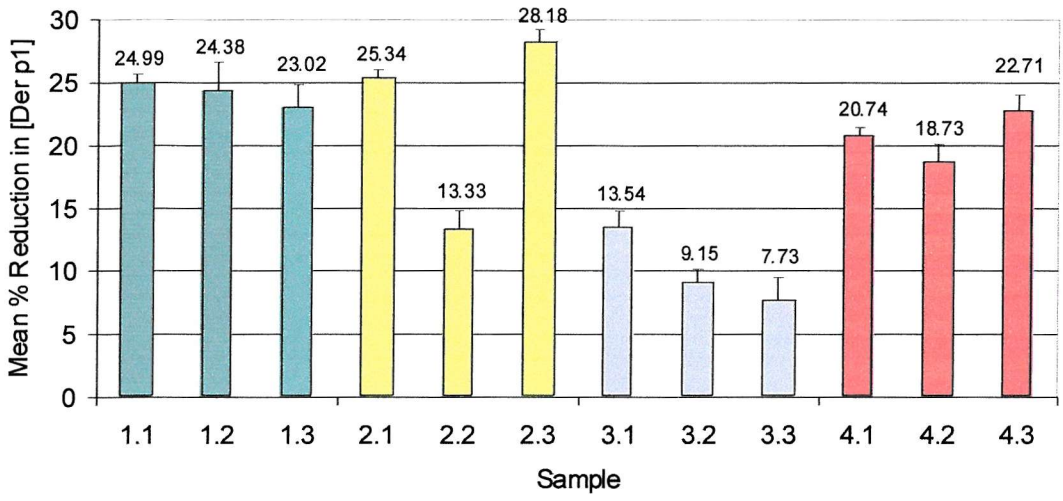


Figure 4.21 **The mean percentage reduction in Der p1 concentration of samples in various positions around the unoccupied, furnished room after 3 weeks exposure to the experimental ion wind generator.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5*. All values are statistically significant. (n= 6; SEM shown).

A pattern can be seen in the percentage reductions observed within each radial sector equidistant from the ioniser. The reductions in samples placed directly in line with the ioniser (i.e., Samples 1.2, 2.2 and 4.2) were always statistically lower than those placed on either side when compared using the Mann Whitney-U test (P<0.05). This suggests that the ion wind forms a divergent plume or bifurcates upon exiting the ioniser thus leaving a lower concentration of corona products in the middle. This effect was not observed in the chamber test, possibly due to the different positions of the samples and the smaller distances involved.

Another explanation for the lower reductions in the samples positioned in line with the ioniser could be due to their relative positions in the room: Samples 1.2 were placed 0.3m in front of the ioniser but below the flow of the ion wind; Samples 2.2 were placed on the floor 1.05m below the centre of the ion wind generator; Samples 4.2 were also placed on the floor but with the desk and chair in line with the ioniser. These sample positions,

particularly those fixed to the floor, could receive less exposure to the corona products than those 0.9m above the floor and fixed horizontally (i.e., Samples 1.1, 1.3, 2.1, 2.3, 4.1 and 4.3).

Whereas the majority of reductions in the samples placed on either side of the room and exposed for one week were approximately equal at 35%, the samples in the third radial sector were much lower at 16.30 ±3.39%, 11.49 ±3.18% and 16.54 ±1.11% (for Sample 3.1, 3.2 and 3.3 respectively). Samples 3.1 and 3.3 were fixed vertically to the walls 0.9m above the floor and 2.4m away from the ioniser. These lower reductions might indicate less capture of the corona products because of their vertical position. It is likely that the other samples, which were fixed horizontally, had more exposure to the active corona product(s) due to precipitation directly onto the foil with the samples. Sample 3.2, although placed 0.9m above the floor on the desk, was positioned with a soft-furnished office chair, with a height of 1m, in between the desk and the ioniser. The chair might have shielded the samples from the corona products.

After two weeks of exposure, the majority of observed reductions in Der p1, shown in *Figure 4.20*, were similar in magnitude to those achieved after one week of exposure. Moreover, there is no time-dependent increase in reductions as Samples 2.3, 3.3, 4.1, 4.2 and 4.3 were all statistically lower than the reductions achieved after only one week in the equivalent positions ($P<0.05$). It is unclear why these reductions were lower after two weeks of exposure although the general patterns noted for the reductions after one week also appear in the samples exposed for two weeks. That is, the samples in a direct line in front of the ioniser were always lower than the samples to the side. Sector 3 again shows the lowest reductions, probably due to the vertical positioning of Samples 3.1 and 3.3 and the positioning of Sample 3.2 behind the chair on the desk.

The reductions observed after three weeks (see *Figure 4.21*) show a different pattern of reductions. Although a time-dependent increase in reductions was not observed, even after three weeks of exposure, the reductions within Sectors 1, 3 and 4 were all relatively similar to each other, i.e., the central samples were not reduced any less than the samples

positioned to the side of the room. Sector 2 however, still showed this pattern. The reductions observed in Samples 1.1, 1.3 and 3.3 were all statistically lower than those observed after one week ($P<0.05$). Samples 2.1, 4.1 and 4.3 were reduced less than those achieved after both a one week and a two week exposure, whereas Sample 1.2 was reduced more ($P<0.05$). A possible reason why the reductions in Sample 1.2 were greater than in previous exposures may be linked to the absence of the pattern noted above. After three weeks of exposure, it is possible that the diffusion of corona products has enabled the samples on the floor in Sector 4, and just below the ioniser in Sector 1, to receive approximately the same quantity of active corona product(s) as those samples positioned to the sides of the room.

4.8.2 ROOM-SCALE EXPOSURE OF SAMPLES TO THE *IONIC BREEZE*™ *SILENT AIR PURIFIER*

The results of this test showed that exposure to the corona products of the *Ionic Breeze*™ *Silent Air Purifier* in the furnished office room led to statistically significant reductions in the Der p1 concentration of the samples. *Figures 4.22, 4.23 & 4.24* show the Der p1 concentrations of the controls and samples in their various positions in the furnished office room after one, two and three weeks exposure respectively. The control concentrations were relatively constant with mean concentrations of 223.35 ±3.56ngml^{-1}, 222.54 ±3.41ngml^{-1} and 225.33 ±4.17ngml^{-1} for the one, two and three week exposures respectively. All samples, except Sample 4.2 after two weeks exposure and Samples 4.1, 4.2 and 4.3 after three weeks exposure, were statistically lower than their controls when compared using the t-test or the Mann Whitney-U test where appropriate ($P<0.05$). The ozone concentration in the room, throughout the three exposure periods, was negligible, although a concentration of 0.025ppm was recorded immediately in front of the ioniser.

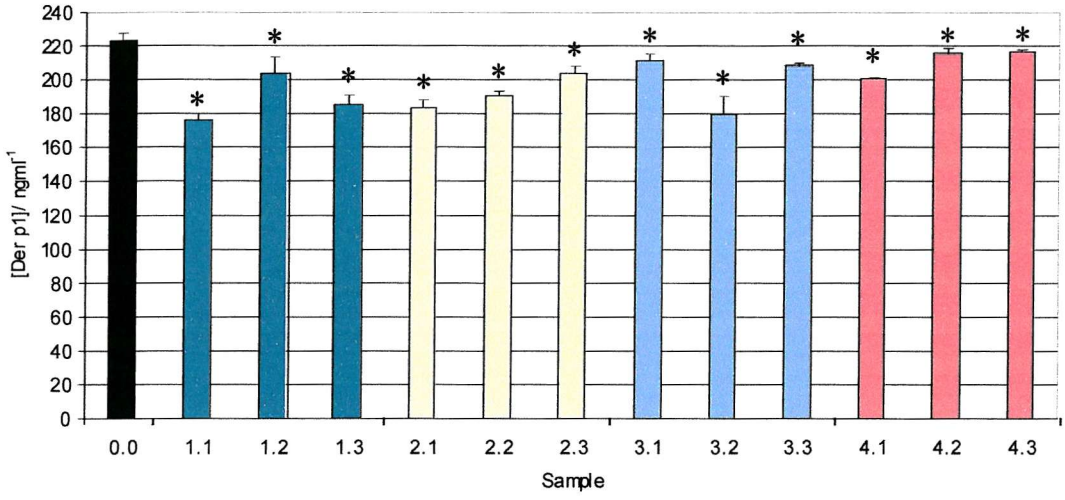


Figure 4.22 **The Der p1 concentrations of the controls and samples in various positions around the unoccupied, furnished room after 1 week exposure to the *Ionic Breeze*™ *Silent Air Purifier* .**
The sample numbers refer to the position in each sector as shown in *Figure 4.5.* Controls (0.0) were exposed to the ambient atmosphere for the equivalent period. * = Statistically lower than the control. (Controls: n=16; Samples: n= 6; SEM shown).

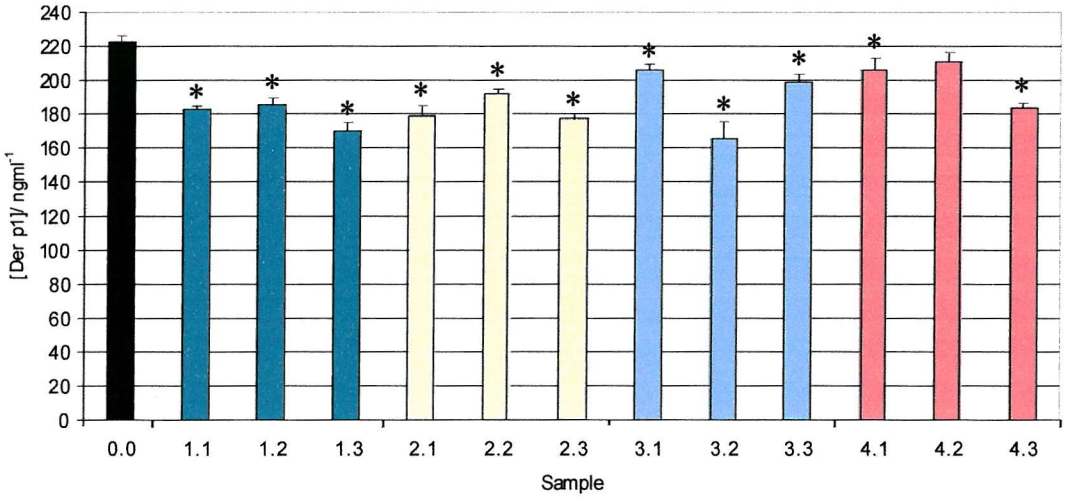


Figure 4.23 **The Der p1 concentrations of the controls and samples in various positions around the unoccupied, furnished room after 2 weeks exposure to the *Ionic Breeze*™ *Silent Air Purifier* .**
The sample numbers refer to the position in each sector as shown in *Figure 4.5.* Controls (0.0) were exposed to the ambient atmosphere for the equivalent period. * = Statistically lower than the control. (Controls: n=16; Samples: n= 6; SEM shown).

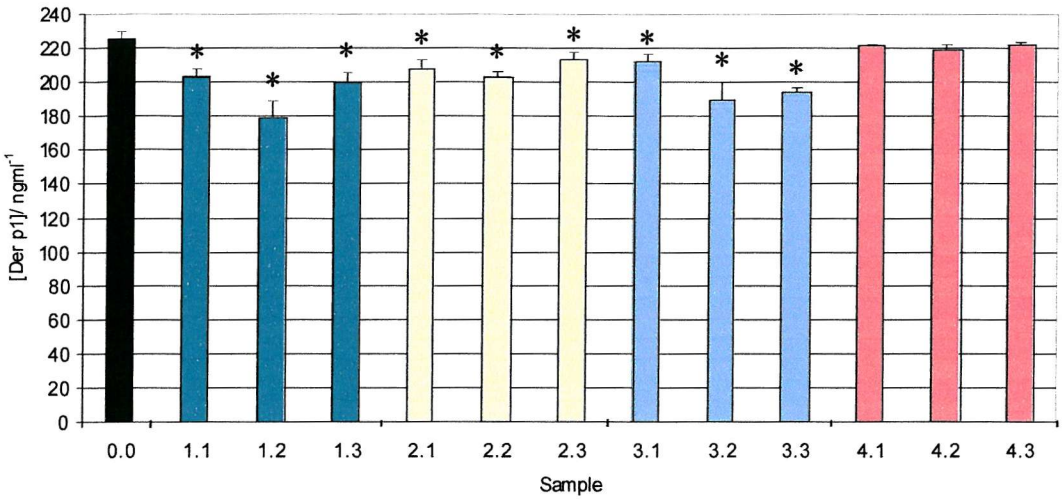


Figure 4.24 **The Der p1 concentrations of the controls and samples in various positions around the unoccupied, furnished room after 3 weeks exposure to the *Ionic Breeze*™ *Silent Air Purifier*.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5*. Controls (0.0) were exposed to the ambient atmosphere for the equivalent period. * = Statistically lower than the control. (Controls: n=16; Samples: n= 6; SEM shown).

Figures 4.25, 4.26 & 4.27 show the mean percentage reductions in Der p1 concentration over the three exposure times. After one week of exposure to the *Ionic Breeze*™ ioniser, reductions in the Der p1 concentration of samples were achieved that ranged from 2.91 ±1.08% in Sample 4.3, 4m distant from the ioniser, to 21.15 ±1.08% 0.3m from the ioniser. Samples 4.2 and 4.3 were reduced the least 4m away from the ioniser, although no relationship between the distance the samples were from the ioniser and the reductions achieved can be seen. The majority of samples were reduced in Der p1 concentration less than those exposed to the experimental nine-pin ion wind generator for the equivalent exposure period even though the commercial ioniser had a faster rate of ion production and a greater velocity ion wind (see *Section 4.6*). Samples 1.1, 1.2, 1.3, 2.1, 2.3, 3.1, 3.3, 4.1, 4.2 and 4.3 all had statistically lower reductions than those achieved with the experimental ioniser (P<0.01); the reduction in Sample 2.2 was not statistically different

and only Sample 3.2 was greater than its equivalent reduction with the experimental ioniser ($P<0.05$).

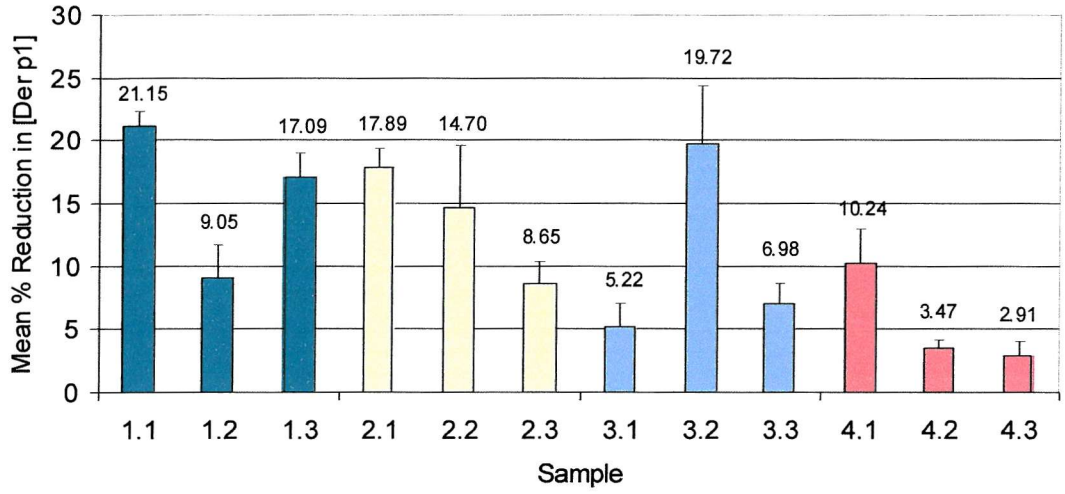


Figure 4.25 **The mean percentage reduction in Der p1 concentration of samples in various positions around the unoccupied, furnished room after 1 week exposure to the *Ionic Breeze™ Silent Air Purifier*.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5*. All values are statistically significant. (n= 6; SEM shown).

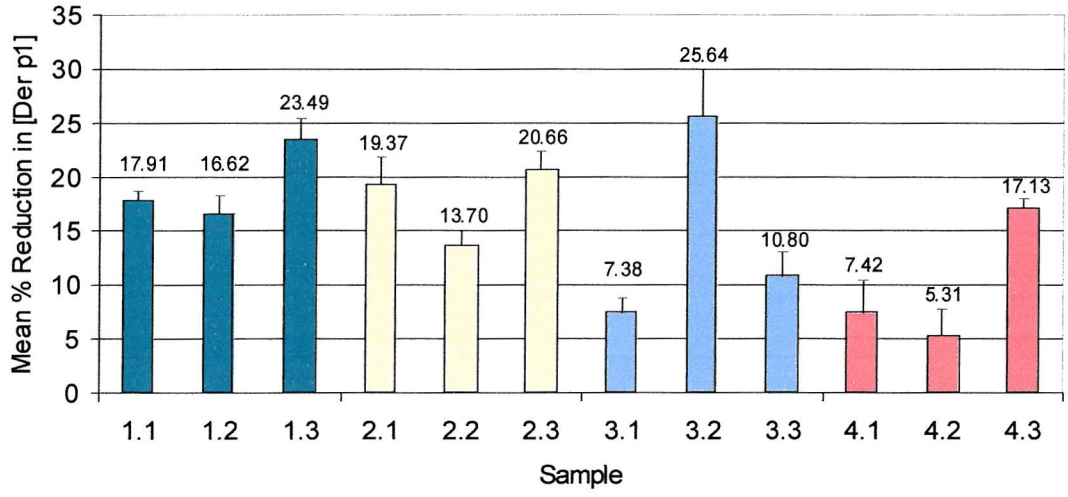


Figure 4.26 **The mean percentage reduction in Der p1 concentration of samples in various positions around the unoccupied, furnished room after 2 weeks exposure to the *Ionic Breeze™ Silent Air Purifier*.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5*. All values are statistically significant except sample 4.2. (n= 6; SEM shown).

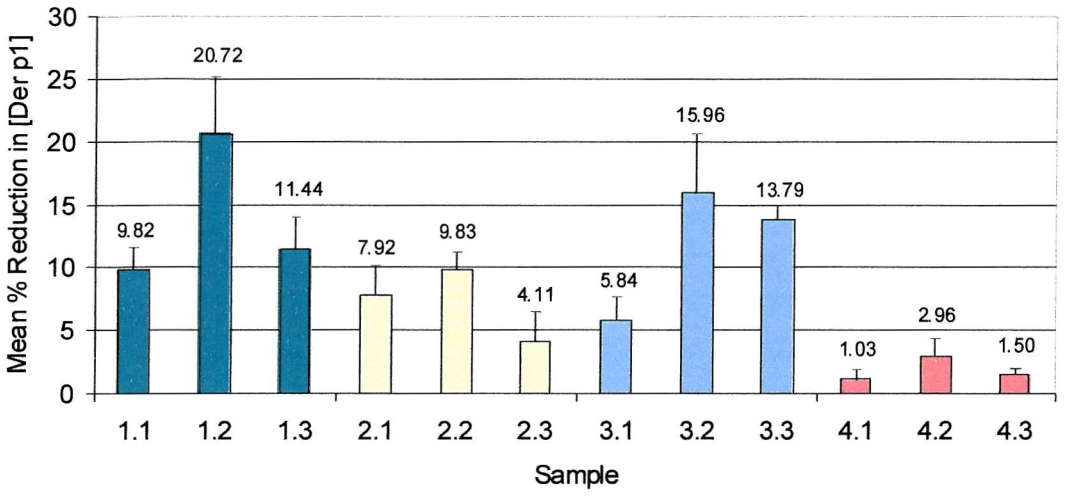


Figure 4.27. **The mean percentage reduction in Der p1 concentration of samples in various positions around the unoccupied, furnished room after 3 weeks exposure to the *Ionic Breeze*™ *Silent Air Purifier*.**
The sample numbers refer to the position in each sector as shown in *Figure 4.5.* All values are statistically significant except samples 4.1 – 4.3. (n= 6; SEM shown).

The pattern described in *Section 4.8.1* concerning the greater reduction of samples placed to either side of the ioniser is only present in Sector 1. It is possible that the release of the ion wind along the length of the three, 478mm long wire electrodes in all directions led to a greater degree of corona product diffusion. Sector 3 also showed a difference in the reductions achieved with the *Ionic Breeze*™ ioniser. Whereas the experimental ioniser caused less reduction in the Der p1 concentration of samples in Sector 3 (possibly due to the vertical positioning of the side samples and Sample 3.2 positioned behind a chair on the desk), the second highest reduction of 19.72 ±4.61% was observed in Sample 3.2 with the commercial ioniser. An explanation for this may reside in the height of the *Ionic Breeze*™ ioniser, which was 338mm (electrode length) taller than the experimental ioniser. This would have enabled the corona products to overcome the shielding effect of the soft-furnished chair in line with the ioniser and Sample 3.2 and so destroy more Der p1 than the corona produced with the nine-pin experimental ioniser.

Figure 4.26 shows the mean percentage reductions in Der p1 concentration of the samples after two weeks exposure to the *Ionic Breeze*™ ioniser. The percentage reductions ranged from 25.64 ±4.36% in Sample 3.2, to the non-significant 5.31 ±2.44% reduction in Sample 4.2. As found in the tests with the experimental ioniser, the majority of the samples' reductions observed after two weeks exposure were similar in magnitude to those achieved after one week of exposure. The reductions in Samples 1.1, 1.3, 2.1, 2.2, 3.1, 3.2, 3.3, 4.1 and 4.2 were not significantly different from the reductions observed after one week ($P>0.05$), and only the reductions in Samples 1.2, 2.3 and 4.3 were significantly greater ($P<0.01$). The pattern of reductions evident in the one and two week exposures with the experimental ioniser only appears in Sector 2 in this test and Sector 3 shows the same general pattern as that described in the one week exposure for the commercial ioniser. Similar to the tests with the experimental ioniser, no time-dependent increases in reductions can be observed.

After three weeks exposure to the *Ionic Breeze*™ ioniser, the reductions observed in the office room (see *Figure 4.27*) ranged from 20.72 ±4.44% in Sample 1.2 to the non-significant 1.03 ±0.86% in Sample 4.1. The reductions achieved after this three week exposure were rarely significantly greater than the reductions achieved with less time exposure. Only the reductions in Sample 1.2 were greater than both the one week and two week exposures ($P<0.05$), and the reductions in Sample position 3.3 were only greater than the one week exposure in the same position ($P<0.05$). Reductions in Samples 2.2, 3.1 and 3.2 were statistically similar to the reductions after one or two weeks exposure, and the reductions in Samples 1.1, 1.3 and 2.1 were statistically less than those achieved in the one or two weeks exposure ($P<0.05$). The reductions observed in Sample 2.3 were less than those observed after the two week exposure.

Unexpectedly, the samples in Sector 4 were not significantly reduced in this three week exposure to the commercial ioniser. Except for the reductions in Sample 4.3 after two weeks exposure and Sample 4.1 after one week of exposure, very little Der p1 was destroyed in samples in this sector 4m from the ioniser. This is the only indication that

the Der p1-destroying efficacy of the commercial ioniser decreases with an increase in distance from the ioniser. This could be due to the greater diffusion of the ion wind with the commercial ioniser than the experimental ioniser; the more diffuse corona products may be captured by the electrically earthed walls and furnishings before they reach the opposite side of the room 4m away. The more diffuse corona products may also be the reason why the pattern observed with the experimental ioniser was not observed in these *Ionic Breeze*™ exposure tests.

The majority of reductions observed in this test were significantly less than those achieved after exposure to the experimental ioniser for the equivalent period. Only the reductions in Sample 3.2 and 3.3 were greater, possibly due to the larger, wire electrodes ($P<0.05$), and the reductions in Sample 1.2 and 2.2 were statistically similar.

It is likely that the other allergens (Der f1, Der p2 and Fel d1), tested in the pin-to-plane corona set-up in *Chapter 3*, will be susceptible to damage by the corona products produced by ionisers. Therefore, ionisers present a novel method for destroying a number of different types of allergens in the domestic environment.

It has been shown that corona products from ionisers are effective at destroying vertically positioned Der p1 samples although, in the room-scale tests, less reduction in Der p1 concentration was achieved in the majority of tests compared with the horizontally positioned samples. This reduction of allergen placed on walls indicates that practical application of corona discharge in the domestic environment would be effective at reducing the significant amounts Fel d1 found on wall surfaces (Wood, Mudd & Eggleston, 1992). The deposition of Fel d1 on walls is due to the aerodynamic properties of the aeroallergen. The larger particulate matter carrying mite allergens do not stay airborne for long periods and so is not deposited on wall surfaces as widely as cat allergen

Although the commercial, wire-to-electrode ionisers produced more ions than the nine-pin, experimental ioniser, in both tests greater reductions were observed after exposure to

the experimental ioniser. However, due to the diffuse release of the ion wind with the wire-to-electrode configurations, the commercial ionisers might be better suited at overcoming the shielding effects of furniture and thereby distributing the corona products in a domestic environment.

4.9 THE EXTENT OF ACTIVE CORONA PRODUCT PENETRATION

The results of this investigation have shown that the corona products responsible for destroying the Der p1 allergen can penetrate certain fabrics and cushion foams although the depth to which they penetrate depend on the design of the foam. *Figure 4.28* shows the Der p1 concentrations in the samples exposed to the nine-pin ion wind generator for 48 hours beneath fabrics and their controls. All of the samples, except those exposed beneath upholstery fabric, were statistically lower in Der p1 concentration than their paired controls when compared using the paired t-test for means, or the Mann Whitney-U test where appropriate ($P<0.05$). The concentration of the controls was relatively constant at 233.71 ±2.41ngml^{-1} (n=48).

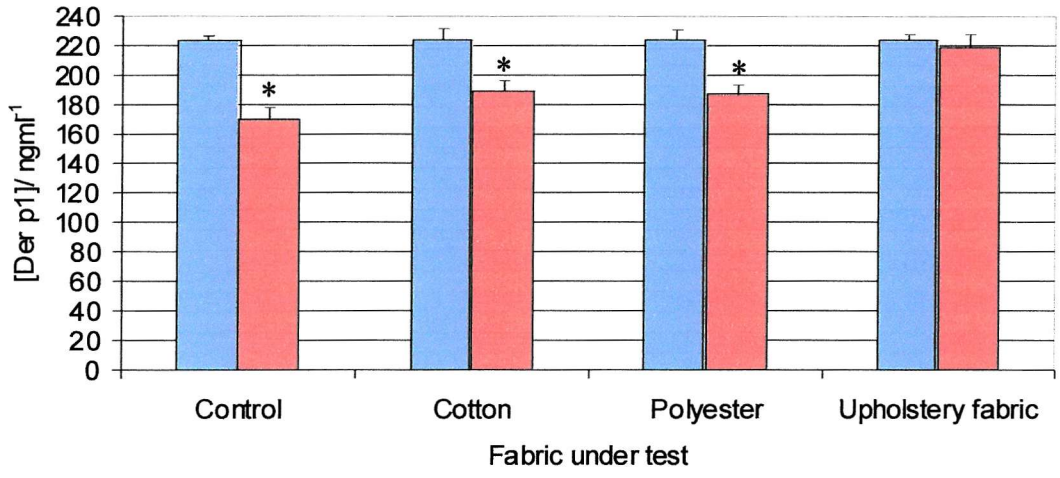


***Figure 4.28* The Der p1 concentrations in the controls and samples exposed to the nine-pin ion wind generator beneath different fabrics.** Samples (□) were exposed to negative corona for 48 hours beneath cotton, polyester or upholstery fabric. Controls (□) were exposed to the ambient atmosphere for the equivalent period. The samples in the positive control test were exposed for 48 hours with no fabric covering (*= statistically lower than the control, n=12, SEM shown).

Figure 4.29 displays the mean percentage reductions achieved in the Der p1 samples exposed to the experimental ioniser under different fabrics. The positive control experiment, with no fabric between the corona source and the samples caused the highest reduction of 23.55 ±2.20%. Lower reductions were recorded after exposure beneath cotton or polyester fabric: 15.70 ±2.27% and 16.91±2.40% respectively. These values were not significantly different from the positive control values or each other when the percentage reductions were compared using the Mann Whitney-U test ($P<0.05$). The samples beneath the upholstery fabric failed to be significantly reduced by the corona products.

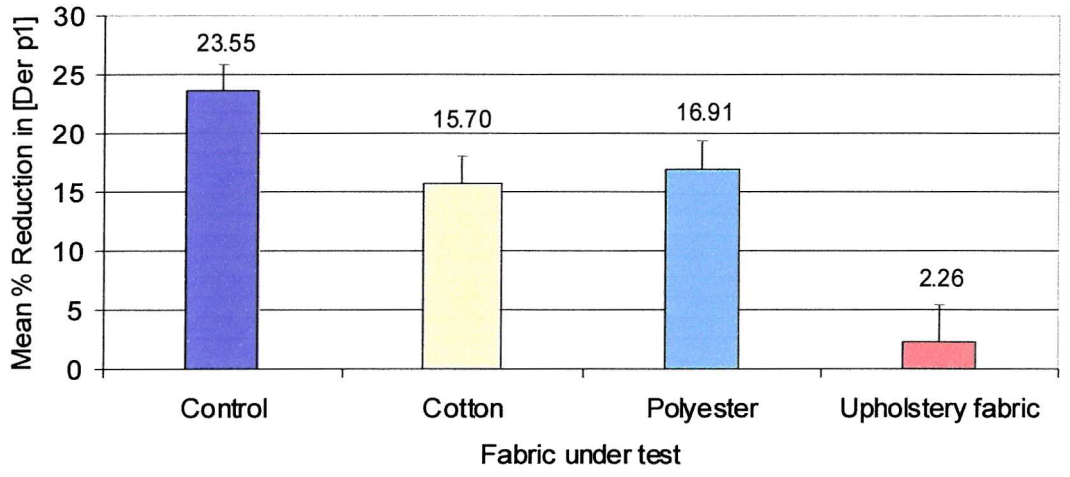


***Figure 4.29* The mean percentage reductions in the samples exposed to the nine-pin ion wind generator beneath different fabrics.**
The samples in the positive control test were exposed for 48 hours with no fabric covering (n=12, SEM shown).

Table 4.1 shows the current recorded in the ground line during these tests. As can be seen, the current was less when fabrics were placed between the ioniser and the samples, although the current recorded was approximately the same for each fabric. Therefore, the insignificant reduction recorded beneath the thicker upholstery fabric cannot be explained by the failure of ions to penetrate the fabric. This suggests that one, or more, of the other

corona products are necessary to destroy the Der p1 allergen. However there was a strong, positive correlation between the current recorded and the magnitude of the percentage reductions (Spearman's rho=0.700, $P<0.01$).

Material	Thickness/ mm	Current/ (10^{-7}) A
Control	0.00	4.5
Cotton	0.25	1.8
Polyester	0.20	1.5
Upholstery fabric	1.05	1.4
Closed cell foam	5	1.6
	10	1.5
	25	1.0
	50	1.2
Open Cell foam	5	2.2
	10	1.9
	25	1.4
	50	1.3

***Table 4.1* The current recorded from the ground line with various fabrics or foams between the ioniser and the ground electrode.**
The two foams were closed cell, expanded polypropylene foam and open cell, reticulated polyester foam.

Figure 4.30 shows the Der p1 concentration of samples exposed beneath different thicknesses of closed cell, expanded polypropylene foam and their controls. The concentration of the controls was relatively constant throughout the test with a mean concentration of 221.31 ±3.68ngml^{-1} (n=60). The concentration of the samples were only significantly less than their paired controls beneath 5mm of closed cell foam ($P<0.05$). No significant reductions were recorded beneath the closed cell foam of any greater thickness. *Figure 4.31* shows the Der p1 concentrations in the samples exposed beneath different thicknesses of open cell, reticulated foam. The concentrations of the controls in this test were constant at 223.26 ±2.94ngml^{-1} (n=60). The samples were statistically lower than the controls beneath foams of up to 10mm thick.

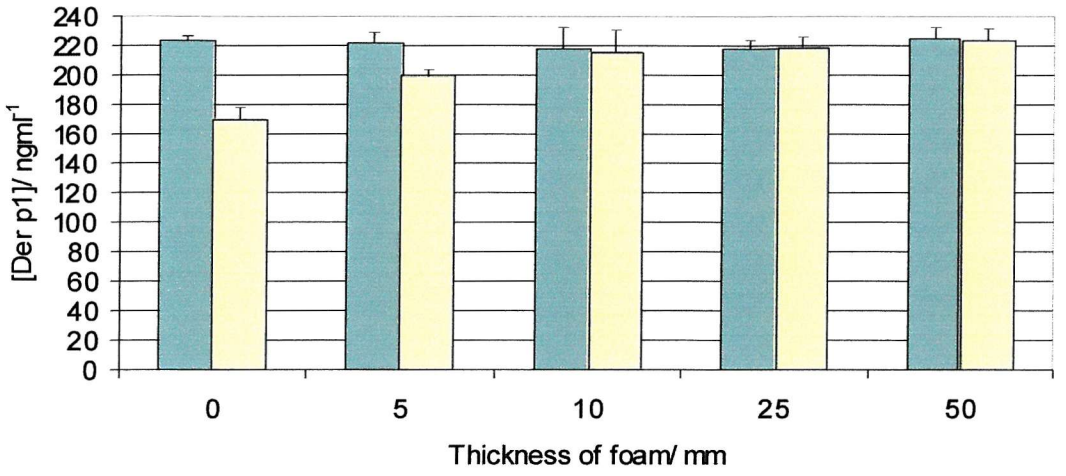


Figure 4.30 **The Der p1 concentrations in the controls and samples exposed to the nine-pin ion wind generator beneath closed cell, expanded polypropylene foam of varying thickness.**
Samples (□) were exposed to negative corona for 48 hours beneath different thicknesses of closed cell, expanded polypropylene foam. Controls (■) were exposed to the ambient atmosphere for the equivalent period. The samples in the positive control test (0mm thickness) were exposed for 48 hours with no foam covering (*= statistically lower than the control, n=12, SEM shown).

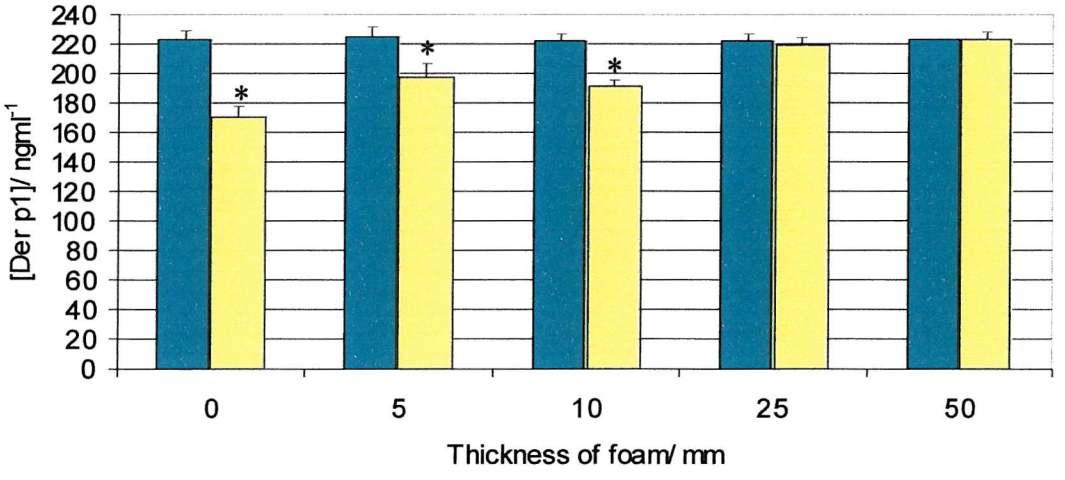


Figure 4.31 **The Der p1 concentrations in the controls and samples exposed to the nine-pin ion wind generator beneath open cell, reticulated foam of varying thickness.**
Samples (□) were exposed to negative corona for 48 hours beneath different thicknesses of open cell, reticulated foam. Controls (■) were exposed to the ambient atmosphere for the equivalent period. The samples in the positive control test (0mm thickness) were exposed for 48 hours with no foam covering (*= statistically lower than the control, n=12, SEM shown).

The mean percentage reductions in Der p1 concentration of the samples exposed to the experimental ioniser beneath different thicknesses of either closed cell, or open cell, foam are shown in *Figure 4.32*. The highest reduction of 23.55 ±2.20% was, predictably, in the absence of any foam in the positive control. The open cell, reticulated polyester foam allowed more ions to pass through at each thickness than the closed cell, expanded polypropylene foam (*Table 4.1*) and also allowed the Der p1 samples to be reduced at a thickness 5mm greater than the closed cell foam. The open cell foam allowed reductions of 11.95 ±2.85% and 13.20 ±4.20% with thicknesses of 5 and 10mm compared with 9.56 ±3.65% and the insignificant 0.37 ±1.49% for the closed cell foam. Neither foam permitted reductions in the Der p1 samples at thicknesses greater than 10mm. Strong, positive correlations exist between the percentage reductions obtained and the current recorded from the ground line (rho=0.703 and 0.793, $P<0.01$ for closed cell and open foam respectively) although no statistical difference exits between the reductions achieved with open cell foam at thicknesses of 5mm and 10mm.

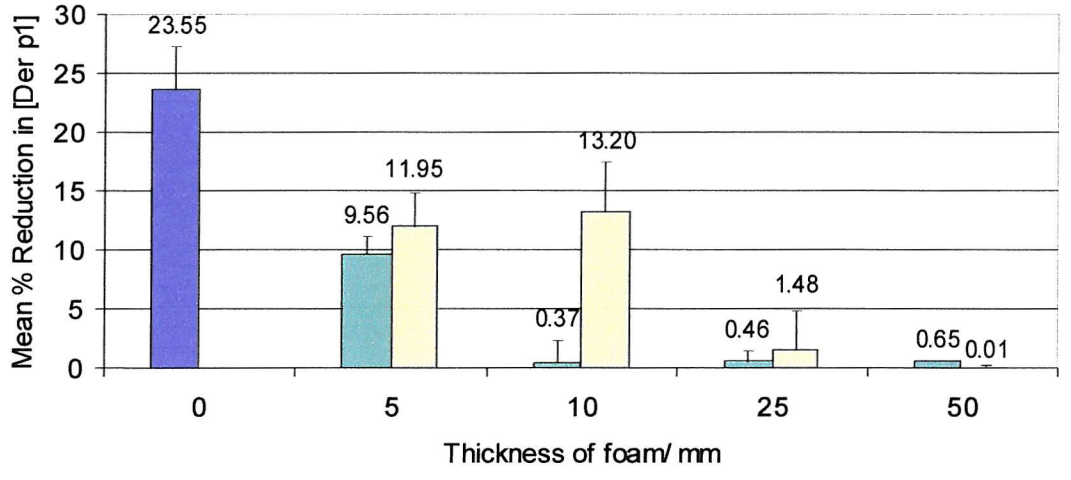


***Figure 4.32* The mean percentage reductions in the samples exposed to the nine-pin ion wind generator beneath different thicknesses of closed, or open, cell foam for 48 hours.**
(■) = Samples in the positive control test exposed for 48 hours with no foam covering, (■) = samples exposed beneath closed cell, expanded polypropylene foam, (□) = samples exposed beneath open cell, reticulated polyester foam (n=12, SEM shown).

These results have shown that the progress of corona products was retarded when fabric or foam was placed between the corona source and the target Der p1 samples. 33% less reduction was observed with cotton than with the positive control, 28% less with polyester and 90% less with upholstery fabric. 59% less reduction was observed with 5mm of closed cell foam and 49% and 44% less reduction was observed with 5mm and 10mm of open cell foam respectively. This shows that the simple method of placing an ioniser so that the ion wind is directed onto the soft furnishing would not significantly reduce the allergen reservoir within. The potential application of using ionisers to reduce the allergen load in the domestic environment would have to be more sophisticated. However, it has been reported that significant amounts of mite and cat allergen can be found on hair and clothing (Siebers, Rains, Fitzharris *et al.*, 1998; Siebers, Patchett, Crane, 1996). This has led to contamination of homes without cats, schools and other public places and caused significant amounts of allergen to be deposited (Chan-Yeung, Manfreda, Dimich-Ward, 1995; Warner, 1992). Placing an ioniser close to garments would lead to the allergen carried on that garment to be destroyed, thereby reducing the risk of inter-building contamination.

Although the tests using ionisers in a chamber and office room (*Sections 4.7 and 4.8*) showed that these alone were sufficient to reduce the surface allergen concentration, another protocol would have to be developed in order to destroy allergen deep within soft furnishings, mattresses etc. Techniques to enable the active corona product(s) to penetrate deep into soft furnishings need to be developed. It is possible that artificially increasing the velocity of the ion wind by the use of fans would enable the corona products to penetrate more deeply. Other methods, such as increasing the quantity of corona products or by increasing the atmospheric pressure outside the furnishing (or reducing the pressure within) might enable these products to penetrate more deeply.

CHAPTER 5: CONCLUSIONS AND FURTHER RESEARCH

The results of the investigations presented here have shown that corona discharge can destroy the major allergens of the European house dust mite Der p1 and Der p2, the major allergen of the American house dust mite Der f1, and the major cat allergen Fel d1.

The investigation into the effect of negative corona discharge on Der p1-carrying faecal particles (*Sections 2.3 & 2.6*) showed that the concentration of Der p1 was reduced although the high degree of concentration variability in the samples and controls resulted in the majority of reductions being insignificant. The investigation into improving the Der p1 concentration of samples (*Section 2.4 & 2.7*) led to a novel method of allergen sample preparation that gave a higher degree of concentration homogeneity and properties that made it possible to conduct detailed investigations into the effect of corona discharge on allergens.

The effect of the amount of allergen present in the sample prior to exposure to negative Trichel corona was investigated in *Sections 3.2 & 3.10*. A negative correlation was observed between the initial concentration of allergen in the sample and the percentage reduction achieved in that sample's concentration. The amount of allergen destroyed per 120 minute exposure with a constant corona current however was calculated to remain relatively constant. Although significant differences were found between the number of destroyed moles of allergen in the tests on Der p1, Der f1, Der p2 and Fel d1, all the allergens had similar mean values for the number of moles of allergen destroyed. The overall mean amount of allergen destroyed for the exposure was calculated as $5.14 \times 10^{-16} \pm 4.76 \times 10^{-17}$mol ($n = 138$).

Negative corona discharge destroyed all allergens tested in a time-dependent manner (*Sections 3.3 & 3.11*). The percentage reduction in allergen concentration after treatment by positive corona was also found to be exposure time-dependent except for Fel d1

whose reductions were negligible with this polarity. Fel d1 had the largest reductions per unit exposure time to negative corona with a 100% reduction recorded after 180 minutes.

The concentrations of the *Dermatophagoides* mite allergens experienced approximately similar reductions after exposure to negative Trichel corona with a maximum reduction of 96.70 ±1.45% recorded after 300 minutes with Der f1. Surprisingly, the heat and pH resistant Group 2 allergen Der p2 was destroyed the most during exposure to positive continuous glow corona with a maximum reduction of 96.42 ±1.69% after 300 minutes exposure.

The percentage reductions in allergen concentration for all allergens followed an exponential growth to maximum relationship, the plateau of which may represent the saturation point where oxidation processes are counterbalanced by decarboxylation processes. Similarities to the surface treatment of polymers (Laurent, Mayoux, Noel *et al.*, 1983) indicate that the possible oxidation of the amino group within peptide bonds could lead to the degradation of the protein into its constituent amino acids. If this is the case then both the conformational, and any linear, epitopes would have been destroyed and would prevent the allergen from renaturing upon the return of the protein's optimum conditions. In *Sections 3.8 & 3.16*, no increase in Der p1 concentration occurred (after exposure to negative corona discharge) over the period of two, six or twelve weeks when stored in buffer solution of the optimum pH. This indicates that the protein didn't renature and so it is more likely that chemical modifications or cleavage of the protein's primary structure occurred during the corona exposure.

The magnitude of the corona current affected the corona's efficacy to destroy allergens (*Sections 3.4 & 3.12*). The relationship between corona current and the percentage reduction in allergen concentration achieved follows an exponential growth to maximum curve. The plateau of this curve may also represent the saturation point of reactions occurring between the active corona products and the protein. Fel d1 had the greatest reductions after exposure to negative corona for 60 minutes reaching 79.08 ±3.28% at 30μA although this allergen maintained its integrity at all positive corona currents tested.

The mite allergens experienced similar reductions in concentration for each current value of both polarities. The similarity in the response of Group 1 allergens may reflect their extensive amino acid homology, although the destruction of Der p2 and Fel d1 indicates that corona products use a broad-spectrum method to destroy allergens, probably affecting the primary structure of the protein.

Negative corona discharge has been shown to be dependent on the relative humidity of the surrounding atmosphere for its efficacy to destroy Der p1 (*Sections 3.5 & 3.13*). Positive corona did not show this dependence and may be due to the negative discharge's tendency to passivation of the anode under low RH leading to no further surface reactions (Goldman, Goldman & Sigmond, 1985). Possible experimental artefacts caused by the aluminium oxides produced by reaction with the corona products have been ruled out (*Sections 3.7 & 3.15*). The aluminium products may have interacted with the allergen protein thus reducing the measurement of allergen concentration directly, or indirectly by interfering with the biological components of the ELISA. However, these effects were not observed.

It has been shown that molecular ozone is not responsible for the allergen-destroying effects of corona discharge (*Sections 3.6 & 3.14*). Further research could be conducted into identifying the active corona species responsible for the broad-spectrum destruction of allergens. This could be performed using atmospheres of different pure gases in combination with biochemical techniques to determine the structural/ chemical alterations after exposure to these different corona products. Identification of the active species could make it possible to enhance an ioniser's production of these species, whilst reducing the production of harmful ozone. This would be extremely beneficial. By increasing the production of active corona products, whilst reducing ozone, for example by heating the corona electrode and modifying the electrode configuration of the ioniser (Liu, Guo, Li *et al.*, 2001), the allergen content of the room could be reduced more efficiently and safely. Alexeff & Kang (1999) have suggested the introduction of a small amount of non-electronegative gas, such as nitrogen, around the corona-generating points of the ioniser to intensify the corona produced. By using this method they have increased

the corona current by a factor of ten whilst reducing ozone production to negligible amounts. Identification of the corona product(s) responsible for the destruction of allergens would be the first logical step to take in order to increase the allergen-destroying efficacy of an ioniser. A trade-off between the maximum amounts of these desired products in the corona current and the unwanted ozone could then be made.

Further research is warranted into the structural changes that occur during, and after, exposure to corona discharge. Biochemical structural studies using western blotting, SDS-PAGE and mass spectrometry could determine whether the protein is cleaved due to the breaking of the covalent peptide bond and/ or the disulphide bridges, which stabilise the protein's precise three-dimensional structure. The possible evolution of CO, CO_2 and H_2 gas from the protein samples could also be measured to test the oxidation/ decarboxylation hypothesis.

Structural studies may also reveal structural or chemical differences in Fel d1, which led to its stability under exposure to positive corona discharge. Because the capture monoclonal antibodies of ELISA recognise different epitopes to those employed by human IgE molecules of the immune system *in vivo*, the experiments presented here do not show whether the IgE epitopes are also corona-sensitive. Using IgE in immunoblotting could be used to determine whether the immune system would recognise allergens post exposure. Particular emphasis could be given to Der p2 due to reports that increased binding occurred after denaturation (Tovey, Ford & Baldo, 1989).

In *Chapter 3* the effect of corona discharge on the major household allergens used a pin-to-plane electrode configuration where the allergen samples were fixed to the planar electrode. With the small interelectrode distance of 15mm, heat may have been transmitted to the sample, which could have contributed to the destruction of the proteins (although Der p2 and Fel d1 have been reported to be stable to heat treatment; Lombardero, Heymann, Platts-Mills *et al.*, 1990; Cain, Elderfield, Green *et al.*, 1998). However, in the experiments presented in *Chapter 4* there is a large distance between the corona source and the Der p 1 samples (up to 4m in the case of the room-scale tests),

which became reduced in concentration. This would indicate that heat is not a contributing factor to the mechanism of allergen-destruction by corona discharge. The number of neutral metastable species would also be decreased with increasing distance due to their short half-life, which range from milliseconds to seconds (Kaufman, 1969; Demuth, 1984).

The significance of the results from the chamber, and room-scale, exposures is that reductions in allergen content can be achieved using ionisers that utilise corona discharge. The ozone from the ionisers is also likely to be acaricidal. Although specific experiments using the HDM have not been reported, ozone is known to kill a number of organisms, including insects (Morar, Suarasan, Budu *et al.*, 1997). With a revised protocol for use, ionisers could be safely used to destroy allergens and also kill house dust mites in the domestic environment thus keeping the allergen levels below the minimum needed to elicit sensitisation and symptoms i.e., below $2\mu gg^{-1}$ of dust (Kuehr, Frischer, Meinert *et al.*, 1994). HDMs in laboratory cultures produce a mean of 20 faecal pellets per day, each pellet containing $10ngml^{-1}$ of Der p1, or 100pg per pellet (Tovey, Chapman & Platts-Mills, 1981). This research has demonstrated a maximum reduction of $211.13ngml^{-1}$ in samples on the far wall in the chamber test after three weeks and $92.50ngml^{-1}$ in Sample 4.3 after one week in the room-scale tests both with the experimental ioniser. Direct comparisons between the amounts of Der p1 destroyed by ionisers and the corresponding number of faecal pellets or mites cannot be made based on the research presented here alone due to the evaporated Der p1 solution on aluminium foil method of preparing the samples. Although this method allows detailed investigation into the effect of electrostatic techniques on allergens, it does not mimic natural conditions.

This investigation has focussed on the Der p1 allergen, however, the results presented in *Chapter 3* demonstrate that destruction of Der f1, Der p2 and Fel d1 by this method could also be possible.

Further research is necessary to determine the benefits of allergen destruction using ionisers under natural conditions in the domestic environment – especially any clinical benefits. Pre-clinical trials could be performed to determine whether the decreases in allergen concentration, caused by the ioniser, is associated with decreased skin or airway reactivity to mite IgE sensitised animals or cells.

It would be unwise to recommend continuous use of present-day commercially available ioniser products to reduce the allergen load in a house, due to the fact that exposure to ozone is harmful – especially to atopic individuals (Peden, Woodrow & Devlin, 1995). It may be acceptable to operate the ionisers while rooms are unoccupied. The development of intensive treatments using corona discharge and carried out by specialists, similar to steam cleaning or treatment by liquid nitrogen could be envisaged. In this way, carpets, soft furnishings and mattresses could be cleaned of allergens and HDMs without exposure to patients or pets.

Clinical trials using placebo and active ionisers for long periods of time in the homes of allergy patients would also need to be performed to determine any clinical benefits. Symptoms and medication requirements could be recorded in the patient's diary during the test period and also skin prick tests and other clinical markers for allergy before, during and after the exposure period could be performed. In order for the results to be meaningful however, outside variables such as season, exercise taken, other methods of cleaning the house, and other possible avenues of contact with allergen should be taken into account.

It would be interesting to compare present treatments used to reduce allergens in the domestic environment with this novel method using corona discharge. Both using the improved technique of allergen sample preparation presented in *Sections 2.4 & 2.7*, and the commonly used method of using sieved house dust, or spent culture medium. Well-controlled experiments could be devised where house dust is sampled from different locations in the domestic environment and tested for allergen content before and after exposure to corona discharge and the other techniques.

The allergen used in this investigation was present on the surface of aluminium foil. Although this may mimic the distribution of Fel d1 well which is found in significant amounts on the surfaces of walls (Wood, Mudd & Eggleston, 1992), the majority of the allergenic reservoir in the domestic environment is found inside mattresses and soft furnishings (Colloff, 1998). The results of the investigation into the extent of corona product penetration (*Section 4.5 & 4.9*) have shown that the corona products responsible for destroying Der p1 do not penetrate far: only 10mm into open cell, reticulated foam used in cushions and 5mm into closed cell, expanded polypropylene foam. However, this could be improved by artificially increasing the velocity of the ion wind, or by other methods, to force the corona products deep into furnishings, analogous to the high pressure needed for steam cleaning to be effective.

In conclusion, corona discharge has been shown to destroy allergens from a number of sources. This important, and highly novel, technique could be used to reduce the allergenic load in the domestic environment.

PUBLICATIONS ARISING FROM THIS WORK

Goodman N, Hughes JF (2001) Electrostatic denaturing of the asthma allergen Der p1. UK Patent Application N^{o}: 0101363.0, filed 19th January 2001.

Goodman N, Hughes JF (2002) Electrostatic denaturing of asthma allergens. UK Patent Application N^{o}:0201406.6, filed 19th January 2002.

Goodman N, Hughes JF (2002) The effect of corona discharge on Der p1. *Clin Exp Allergy* **32(4)**: 515-9.

Goodman N, Hughes JF (2002). Improving allergen concentration homogeneity for investigating the effect of corona discharge on Der p1 allergen. *J Electrostatics* **56(1)**: 43-53.

Goodman N, Hughes JF. Long-range destruction of Der p1 using experimental and commercially available ionisers. *Clin Exp Allergy*, in press.

Goodman N, Hughes JF. Novel destruction of domestic cat and house dust mite allergen by corona discharge. *J Allergy Clin Immunol*, under review.

Goodman N, Hughes JF. *In situ* destruction of the major house dust mite allergen Der p1 using an experimental and a commercially available ioniser. *J Electrostatics*, under review.

APPENDIX: STATISTICAL ANALYSIS

PARAMETRIC AND NON-PARAMETRIC TESTS

Statistical tests are either parametric or non-parametric. The conditions for using parametric tests are more rigorous than for non-parametric tests. Data for use in a parametric test should be on an interval or ratio scale of measurement. To use a parametric test the data must also be distributed normally and the variances must be homogeneous. Parametric tests compare means and variances of sample data.

Non-parametric tests can be used to compare nominal, ordinal level or interval scale data. They are suitable for data that are counts of things and for derived data such as proportions and percentages. Non-parametric tests compare the medians of data and very few assumptions are made about the distribution underlying the data and, in particular, it is not assumed to be a normal distribution, or that they have homogeneous variances.

NORMAL DISTRIBUTION AND STANDARD DEVIATION

A normal distribution is when data are dispersed fairly symmetrically either side of the mean with very few extremely high or low values. To test for a normal distribution it is usual to determine whether 70% of the data lies within one standard deviation of the mean. Standard deviation is a measure of the spread of the data around the mean (Fowler & Cohen, 1990). It is calculated by finding the positive square root of the variance:

$$s = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

Where s is the standard deviation from the mean, x is the value of an observation, $\bar{x}$ is the sample mean and n is the number of observations. If the data are normal, the t-test is the better test to use: by using the assumption of normality the test of the mean can be made which gives the most precise results for the available number of observations. But for

non-normal data, the data may be transformed so that the data becomes normal and the t-test applies, or the data may be expressed in rank form and use an example of the non-parametric rank-sum test, such as the Mann Whitney-U test (Clarke & Cooke, 1992)

STANDARD ERROR OF THE MEAN

The standard error of the mean (SEM) is a representation of the spread of data around the sample mean and is often quoted as (Clarke & Cooke, 1992). It is calculated by:

$$\text{SEM} = \frac{s}{\sqrt{n}}$$

Where s is the standard deviation and n is the number of observations.

The standard error of the mean is a measure of the reliability of an estimate of the sample mean, equating to a 68% confidence that the mean falls within one standard error of the mean (Bryars, 1983). The data presented in these investigations show the sample mean and its standard error.

F-TEST FOR COMPARISON OF VARIANCES

In order to use a parametric test to determine whether the means of two sets of data are significantly different, the variances of the two data sets must be checked to ensure that they are similar. If the variances are different, any significant difference calculated by, for example by the t-test (see below), may be recording a difference between the variances rather than the means. The variance of a sample can be calculated by squaring the standard deviation. Two variances can then be compared using an F-test. The Null hypothesis (H_o) in a test for a difference between sample means is therefore:

H_o: two samples are drawn from populations with identical means and variances.

If the outcome of a parametric test suggests the rejection of this null hypothesis, it is necessary to eliminate the possibility that this is due to a difference between variances, rather than a difference between means. The F-test is a simple test to discover whether the difference between the variances is so small that it can be ignored. It is usual to carry out an F-test before testing for a difference between means with, for example, a t-test. If the outcome of the test suggests that the variances are not similar a parametric test for the difference between means cannot be validly applied. The F-test null hypothesis is as follows:

$$H_o: \frac{s_1^2}{s_2^2} = F = 1$$

Where s_1^2 is the variance of sample 1 and s_2^2 is the variance of sample 2. The tabulated critical values are all greater than 1, therefore the test is completed with the greater variance divided by the smaller variance. The degrees of freedom are $(n_1\text{-}1)$ and $(n_2\text{-}1)$ where n_1 and n_2 are the number of observations in samples 1 and 2 respectively. The outcome of the F-test should be compared with the tabulated F-values (P=0.05) for the given degrees of freedom which are published in the relevant statistical tests (for example, Fowler and Cohen, 1990). Values below the critical F-values agree with the null hypothesis. Therefore, the samples can be validly compared by a parametric test to compare means, as the variances of the samples are not significantly different.

T-TEST FOR COMPARISON OF MEANS

The t-test is a parametric test for comparing the means of two samples with less than 30 observations. The number of observations in each sample may be different. The t-value is calculated as follows:

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\left(\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}\right)\left(\frac{n_1 + n_2}{n_1 n_2}\right)}$$

Where the degrees of freedom are $(n_1 + n_2) - 2$. The calculated t-value can be compared to a table of critical t-values at $P=0.05$, for the specified degrees of freedom (Fowler & Cohen, 1990). If the calculated value is greater than the critical t-value, the sample means are significantly different ($P \geq 0.05$). Results are presented as P-values, which show the probability of the samples being different. A t-test can also be performed using the statistical analysis facility of *Microsoft Excel*). t-tests were performed throughout this investigation on the parametric allergen concentration data. In cases where the variance was greater than the mean, and failed to become normal after transformation, the non-parametric Mann Whitney-U test was used.

MANN WHITNEY-U TEST

The Mann Whitney-U test is a non-parametric test for comparing the medians of two unmatched samples. It may be used with as few as four observations in each sample, and the sample sizes may be unequal. As the test is non-parametric it is suitable for use with data that are not normally distributed, therefore it can be used to compare percentages as used throughout this investigation. A test statistic U is calculated that can be compared to tabulated critical values (Fowler & Cohen, 1990).

All of the observations of the two samples to be compared are listed in ascending order. The samples are distinguished by underlining the values of the second data set. These observations are then assigned ranks. The ranks of each sample are then summed.

R_1 = sum of ranks of sample 1

R_2 = sum of ranks of sample 2

The test statistics U_1 and U_2 are then calculated.

$$U_1 = n_1 n_2 + \frac{n_2 (n_2 + 1)}{2} - R_2$$

$$U_2 = n_1 n_2 + \frac{n_1 (n_1 + 1)}{2} - R_1$$

The smaller of the two U values is then selected and compared to the critical value in the tabulated values for the appropriate values of n_1 and n_2. If the smallest value of U is less than the critical value there is a statistically significant difference between the medians. Results are presented as P-values, which show the probability of the samples being different. A Mann Whitney-U test can also be performed using specialist software, such as *SPSS*.

SPEARMAN RANK CORRELATION

The Spearman Rank Correlation involves the calculation of a coefficient r_s (Spearman's rho) which is a measure of the degree of correlation of two variables (Clarke & Cooke, 1992). The two paired sets of observations consisting of interval data are listed in a table and ranked. The difference (d) between the ranks of sample 1 and sample 2 is calculated for each pair of samples. d^2 is then calculated for each pair of samples and summed to obtain Σd^2. The calculation of r_s is then conducted as follows:

$$r_s = 1 - \left[\frac{6\Sigma d^2}{n^3 - n}\right]$$

Where n is the number of units in a sample and d is the difference between ranks. The r_s value can then be compared to an appropriate table of values for the specified n value (Fowler & Cohen, 1990). If the calculated value exceeds the critical value at the appropriate n value at P=0.05, the correlation is significant.

Spearman rank correlations were performed in *Chapter 3* to determine whether the percentage reduction achieved in allergen concentration correlated with the variable under test, such as exposure time or value of corona current. Spearman Rank correlations were also performed in *Chapter 4* to determine whether the percentage reducion achieved in Der p1 concentration in the Chamber exposure tests correlated with exposure time.

The Spearman Rank Coefficient can be calculated using specialist software, such as *SPSS*.

ONE-WAY KRUSKAL-WALLIS NON-PARAMETRIC ANOVA

The Kruskal-Wallis test is a simple non-parametric test to compare between the medians of three or more samples to determine if the samples have come from different populations. Observations may be interval scale measurements, counts of things, derived variables or ordinal ranks In order to perform the Kruskal-Wallis test, the distributions of the data do not have to be normal and the variances do not have to be equal, if there are only three samples, more than five data points per sample should be used. The sample sizes do not have to be equal. The test statistic K is calculated using the following formula:

$$K = \left[\Sigma(R^2/n) \cdot \frac{12}{N(N+1)}\right] - 3(N+1)$$

Where n is the number of observations in each sample, N is the total number of observations, and R is the sum of the ranks of the observations. K can then be compared with the tabulated distribution of χ^2 (Fowler & Cohen, 1990). If a significant difference is found between the samples then there is a difference between the highest and lowest median. Results are presented as *P*-values, which show the probability of their being a difference between the samples. The Kruskal-Wallis test was used on the number of moles of allergen destroyed data (*Section 3.10*), the percentage reductions in Fel d1 concentration after exposure to positive corona discharge (*Section 3.11*), and the percentage reduction data from the renaturing investigation (*Section 3.16*).

COEFFICIENT OF VARIATION

When comparing the variability in samples from populations with different means, the coefficient of variation (CV) is used. This is the ratio of the standard deviation to the

mean, usually expressed as a percentage by multiplying by 100. This was used to compare the degrees of variation in the Der p1 concentration of samples after different methods of sample preparation and distribution (*Section 2.7*).

COEFFICIENT OF DETERMINATION

The coefficient of determination (R^2) represents the proportion of variation in the dependent variable that has been explained, or accounted for, by the regression line. The value of R^2 may vary from zero to one. A coefficient of determination of zero indicates that none of the variation in y is explained by the regression equation; whereas an R^2 value of one indicates that 100% of the variation of y has been explained by the regression line. In order to calculate R^2, the equation below is used:

$$R^2 = \frac{(\Sigma xy - n\bar{x}\bar{y})^2}{(\Sigma x^2 - \bar{x}\Sigma x) \,.\, (\Sigma y^2 - \bar{y}\Sigma y)}$$

R^2 gives an indication of the proportion of total variation that is explained by the regression line. Thus R^2 is a relative measure of the 'goodness' of fit of the observed data points with the regression line. Some applications require R^2 of at least 0.7 (that is 70% of the total variation in the observed values of y is explained by the observed values of x). An $R^2<0.25$ would never be acceptable (*Parametric Estimating Initiative (PEI)Parametric Estimating Handbook*). R^2 values in this investigation were used throughout *Chapter 3*, and were performed using the specialist software, *SigmaPlot*.

REFERENCES

Alexeff I & Kang WL (1999) An enhanced ionizer. *IEEE TRANS PLASMA SCI* **27**: 32-3.

Arlian LG (1989) Biology and ecology of house dust mites, *Dermatophagoides* spp. And *Euroglyphus* spp. *Immunology and Allergy Clinics of North America* **9**: 339-56.

Arlian LG (1992) Water balance and humidity requirements of house dust mite *Dermatophagoides* species and *Euroglyphus* species. *Exp & Appl Acarology* **16**: 15-35.

Baldo BA, Ford SA, Tovey ER (1989) Toward a definition of the complete spectrum and rank order of importance of allergens from the house dust mite: *Dermatophagoides pteronyssinus*. *Adv Biosci* **74**: 13-31.

Ball DJ (1980) Asthma and air ionisation. *Lancet*: 1251-2

Bascom R, Naclerio RM, Fitzgerald TK, Kagey-Sobotka A & Proud D (1990) Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. *Am Rev Respir Dis* **138**: 414-20.

Bastien F, Haug R, Lecuiller M (1975) Simulation sur ordinateur de l'évolution temporelle des ions négatifs de l'air. Application au cas de la décharge couronne negative. *J Chim Phys* **72**: 105-11.

Bates DV, Sizto R (1987) Air pollution and hospital admissions in Southern Ontario: the acid summer haze effect. *Environ Res* **43**: 317-31.

Ben-Dov I, Amirav I, Shochina M, Amitai I, Bar-Yishay E, Godfrey S (1983) Effect of negative ionisation of inspired air on the response of asthmatic children to exercise and inhaled histamine. *Thorax* **38**: 584-88.

Biliotti G, Passaleva A, Romagnani S, Ricci M (1972) Mites and house dust allergy. I. Comparison between house dust and mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) skin reactivity. *Clin Allergy* **2**: 109-13.

Bochner BS, Undem BJ, Lichtenstein LM (1994) Immunologic aspects of asthma. *Ann Rev Immunol* **12**: 295-335.

Briggs D (1983) *Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy*, Briggs D, Seah MP (eds), Chapter 9, Wiley, Chichester.

Bryars DA (1983) *Advanced Level Statistics*. Unwin Hyman Ltd., London.

Burr ML, Dean BV, Merrett TG, Neale E, St Leger AS, Verrier-Jones ER (1980) Effects of anti-mite measures on children with mite-sensitive asthma: a controlled trial. *Thorax* **35**: 506-12.

Cain G, Elderfield AJ, Green R, Smillie FI, Chapman MD, Custovic A, Woodcock A (1998) The effect of dry heat on mite, cat, and dog allergens. *Allergy* **53**: 1213-5.

Chan-Yeung M, Manfreda J, Dimich-Ward H, Lam J, Ferguson A, Warren P, Simons E, Broder I, Chapman MD, Platts-Mills TAE, Becker A (1995) Mite and cat allergen levels in homes and severity of asthma. *Am J Respir Crit Care Med* **152**: 1805-11.

Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE (1988) Monoclonal antibodies to the major feline allergen, Fel dI: II. Single step affinity purification of Fel d1, N-terminal sequence analysis and development of a sensitive two-site immunoassay to assess Fel dI exposure. *J Immunol* **140**: 361-7.

Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TAE (1987) Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens *Der p* 1 and *Der f* 1, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* **80**: 184-94.

Charpin C, Mata P, Charpin D, Lavant MN, Allasia C, Vervloet D (1991) Fel d I allergen distribution in cat fur and skin. *J Allergy Clin Immunol* **88**: 77-82.

Chew GL, Higgins KM, Milton DK, Burge HA (1999) The effects of carpet fresheners and other additives on the behaviour of indoor allergen assays. *Clin Exp Allergy* **29**: 470-7.

Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR (1990) Isolation of cDNA for the major mite allergen *Der p* II by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* **91**: 118-23.

Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, Turner KJ (1988) Sequence analysis of cDNA coding for a major house dust mie allergen *Der p* I. *J Exp Med* **167**: 175 – 82.

Clark RP (1974) Skin scales among airborne particles. *J Hyg (Camb)* **72**: 47-51.

Clarke GM, Cooke D (1992) *A basic course in statistics*. Third edition, Edward Arnold, London.

Colloff MJ (1986) Use of liquid nitrogen in the control of house dust mite populations. *Clin Allergy* **16**: 41-7.

Colloff MJ (1998). Distribution and abundance of dust mites within homes. *Allergy* **53 (Suppl 48)**: 24-7.

Colloff MJ, Ayres J, Carswell F, Howarth PH, Merrett TG, Mitchell EB, Walshaw MJ, Warner JO, Warner JA, Woodcock A (1992) The control of allergens of dust mites and domestic pets: a position paper. *Clin Exp Allergy* **22 (Suppl 2)**: 1-28.

Colloff MJ, Taylor C, Merrett TG (1995) The use of domestic steam cleaning for the control of house dust mites. *Clin Exp Allergy* **25**: 1061-6.

Cross JA (1987) *Electrostatics: principles, problems and applications.* Adam Hilger, Bristol.

Custovic A, Simpson A, Chapman MD, Woodcock A (1998) Allergen avoidance in the treatment of asthma and atopic disorders. *Thorax* **53**: 63-72.

Custovic A, Simpson A, Pahdi H, Green RM, Chapman MD, Woodcock A (1998) Distribution, aerodynamic characteristics, and removal of the major cat allergen Fel d1 in British homes. *Thorax* **53**: 33-8.

Dabrowski AJ, van der Brempt X, Soler M, Seguret N, Lucciani P, Charpin D, Vervolet D (1990) Cat skin as an important source of Fel d1 allergen. *J Allergy Clin Immunol* **86**: 462-5.

de Blay F, Chapman MD, Platts-Mills TAE (1991) Airborne cat allergen (Fel d I). Evironmental control with the cat *in situ*. *Am Rev Respir Dis* **143**: 1334-9.

de Blay F, Heymann PW, Chapman MD, Platts-Mills TAE (1991) Airborne dust mite allergens: Comparison of group II allergens with group I mite allergen and cat-allergen *Fel d* I. *J Allergy Clin Immunol* **88**: 919-26.

de Boer R, van der Hoeven WA, Stapel SO (1995) The decay of house dust mite allergens *Der p* I and *Der p* II, under natural conditions. *Clin Exp Allergy* **25**: 765-70.

de Lucca S, Spovik R, O'Meara TJ, Tovey ER (1999) Mite allergen (Der p1) is not only carried on mite feces. *J Allergy Clin Immunol* **103**: 174-5.

Demuth O (1984) Traitement de surface de fibres synthetiques (poly(ethylene terephtalte)) et naturelles (cotton) par plasmas de type decharge couronne. Doctoral Thesis, L'Universite Pierre et Marie Curie, Paris.

Devlin RB, Mc Donnell WF, Mann R, Becker S, House DE, Schreinmachers D, Koren HS (1991) Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am J Respir Cell Mol Biol* **4**: 72-81.

Dybendal T, Vik H, Elsayed S (1990) Dust from carpeted and smooth floors – III. Trials on denaturation of allergenic proteins by household cleaning solutions and chemical detergents. *Ann Occup Hyg* **34**: 215-29.

Enberg RN, Shamie SM, McCullough J, Ownby DR (1993) Ubiquitous presence of cat allergen in cat free buildings: probable dispersal from human clothing. *Ann Allergy* **70**: 471-4.

Field RC, Schulte HF Jr, Mikat DR, Patterson R & Sheldon R (1961) The effect of artificial unipolar air ionisation on hypersensitivity phenomena. *Univ Mich Med Bull* **27**: 269- 275.

Folinsbee LJ, Mc Donnell WF, Horstman DH (1988) Pulmonary function and symptom responses after 6.6 hour exposure to 0.12ppm ozone exposure. *J Air Pollut Control Assoc* **38**: 28-35.

Ford AW, Rawle FC, Lind P, Spieksma FT, Lowenstein H, Platts-Mills TAE (1985) Standardization of *Dermatophagoides pteronyssinus*: assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Appl Immunol* **76**: 58-67.

Fowler J, Cohen L (1990) *Practical statistics for field biology*. John Wiley & Sons, Chichester.

Gaynor PT, Hughes JF (1998) Electrostatic charge characteristics of Der p1 allergen-carrying particles and the house dust mite *Dermatophagoides pteronyssinus*. *Med Biol Eng Comput* **36**: 351-54.

Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE (1993) Sensitisation and exposure to indoor allergens as a risk factor for asthma among patients presenting to hospital. *Am Rev Respir Dis* **147**: 573-8.

Glinert R, Wilson P, Wedner HJ (1990) *Fel d* I is markedly reduced following sequential washing of cats (abstract). *J Allergy Clin Immunol* **85**: 327.

Goldman M, Goldman A, Sigmond RS (1985) The corona discharge its properties and specific uses. *Pure & Appl Chem* **57**: 1353-62.

Green WF, Nicholas NR, Salome CM, Woodcock AJ (1989) Reduction of house dust mites and mite allergens: effects of spraying carpets and blankets with Allersearch DMS, an acaricide combined with an allergen reducing agent. *Clin Exp Allergy* **19**: 203-7.

Hart BJ (1998) Life cycle and reproduction of house-dust mites: environmental factors influencing mite populations. *Allergy* **53 (Suppl 48)**: 13-7.

Held B, Peyrous R (1999) Physical and chemical studies of corona discharges in air. *Czech J Phys* **49**: 301-20.

Herbert CA, King CM, Ring PC (1995) Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p1. *Am J Respir Cell Mol Biol* **12**:369-78.

Hewitt CRA, Brown AP, Hart BJ, Pritchard DI (1995) A major house-dust mite allergen disrupts the immunoglobulin-E network by selectively cleaving CD23 – innate protection by antiproteases. *J Exp Med* **182**: 1537-44.

Heymann PW, Chapman MD, Platts-Mills TAE (1986) Antigen *Der f* I from the dust mite *Dermatophagoides farinae*: Structural comparison with *Der p* I from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* **137**: 2841-7.

Hill MR (1998) Quantification of house-dust-mite populations. *Allergy* **53 (Suppl 48)**: 18-23.

Holgate ST, Church MK, Lichtenstein LM (2000) *Allergy*. Second edition, Mosby, London.

Ichikawa S, Hatanaka H, Yuuki T, Iwamoto N, Kojima S, Nishiyama C, Ogura K, Okumara Y, Inagaki F (1998) Solution structure of Der f2, the major mite allergen for atopic diseases. *J Biol Chem* **273**: 356-60.

Jerrim KL, Hughes JF, McKechnie MT (2001) Electrostatic enhancement of dust and allergen removal from carpets. *J Electrostatics* **53**: 39-51.

Jones DP, O'Connor SA, Collins JV, Watson BW (1976) Effects of long term ionised air treatment on patients with bronchial asthma. *Thorax* **31**: 428-32.

Kamphuis IG, Drenth J, Baker EN (1985) Thiol proteases: comparative studies based on the high resolution structures of papain and actinidin, and on amino acid sequence information for Cathepsins B and H, and stem bromelain. *J Mol Biol* **182**: 317-29

King TP (1976) Chemical and biological properties of some atopic allergens. *Adv Immunol*: **23**: 77-105.

King CM, Brennan S, Thompson PJ (1998) Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol* **161**: 3645-51.

Koren HS, Devlin RB, Graham DE, Mann R, Mc Gee MP, Horstman DH, Kozumbo WJ, Becker S, House DE, Mc Donnell WF, Bromberg PA (1989) Ozone-induced inflammation in the lower airways of human. *Am Rev Respir Dis* **139**: 407-15.

Kornbleu IH, Griffin JE (1955) Artificial air ionisation in physical medicine, preliminary report. *Amer J Phy Med* **34**: 618-24.

Kornbleu IH, Piersol GM, Speicher FP (1958) Relief from pollinosis in negatively ionised rooms. *Amer J Phys Med* **37**: 18-25.

Korsgaard J (1998a) Epidemiology of house-dust mites. *Allergy* **53 (Suppl 48)**: 36-40.

Korsgaard J (1998b) House dust mites and asthma. A review on house-dust mites as a factor for mite asthma. *Allergy* **53 (Suppl 48)**: 77-83.

Kort HSM, Kniest FM (1994) Four year stability of Der p1 in house dust under simulated domestic conditions *in vitro*. *Allergy* **49**: 131-3.

Kreuger AP (1962) Air ions and physiological function. *J Gen Physiol* **45**: 233-41.

Kreuger AP, Smith RF (1960) The biological mechanism of air ion action: negative ion effect on the concentration and metabolism of 5-HT in the mammalian respiratory tract. *J Gen Physiol* **44**: 269-72.

Kuehr J, Frischer T, Meinert R, Barth R, Forster J, Schraub S, Urbanek R, Karmaus W (1994) Mite allergen exposure is a risk for the incidence of specific sensitisation. *J Allergy Clin Immunol* **94**: 44-52.

Laurent C, Mayoux C, Noel S, Sinisuka NI (1983) A study of emission lines from electrical trees. *IEEE Trans Electr Ins* **18**: 125-30.

Lécuiller M, Julien R, Pucheault J (1972) Étude par voie chimique des ions produits par décharge couronne. *J Chim Phys* **9**: 1353-9.

Lioy PJ, Vollmuth TA, Lippman M (1985) Persistence of peak flow decrement in children following ozone exposures exceeding the national ambient air quality standard. *J Air Pollut Control Assoc* **35**: 1068-71.

Lipin I, Gur I, Amitai Y, Amirav I, Godfrey S (1984) Effect of positive ionisation of inspired air on the response of asthmatic children to exercise. *Thorax* **39**: 594-6.

Lippman M (1989) Health effects of ozone: a critical review. *J Air Pollut Control Assoc* **39**: 672-95.

Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD (1990) Conformational stability of B cell epitopes on group I and group II *Dermatophagoides* spp. allergens. Effect of thermal and chemical Denaturation on the binding of Murine IgG and human IgE antibodies. *J Immunol* **144**: 1353-60.

Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD (1989) A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. Allergens, *Der p*I and *Der f*I. *J Immunol Meths* **118**: 227-35.

Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE (1990) Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*):

measurements using cascade impactor, liquid impactor, liquid impinger, and a two-site monoclonal antibody assay for *Fel d* I. *Am Rev Respir Dis* **141**: 361-7.

Lui L, Guo J, Li J, Sheng L (2001) The effect of wire heating and configuration on ozone emission in a negative ion generator. *J Electrostatics* **48**: 81-91.

Mitchell EA, Elliot RB (1980) Controlled trial of an electrostatic precipitator in childhood asthma. *Lancet* **ii**: 559-61.

Miyoshi Y, Hosokawa T, Sakai O (1964) cited in:
Sigmond RS (1978) Corona Discharges, in Meek (ed.) *Corona Discharges. Electrical Breakdown in Gases*, Ch4: 319-84, Wiley, New York.

Miyamoto T, Oshiwa S, Mizimo K, Sasa M, Ishizaki T (1969) Cross-antigenicity among six species of dust mites and house dust antigens. *J Allergy Clin Immunol* **44**: 228-38.

Morar R, Suarasan I, Budu S, Ghizdavu I, Porca M, Dascalescu L (1997) Corona discharge effects on some parasitical insects of cultured plants. *J Electrostatics* **40&41**: 669-73.

Morley M (1993) Immunopharmacology of asthma. *Trends in Pharm Sci* **14**: 275-85.

Mudd JB, Leavitt R, Ongun A, McManus TT (1969) Reaction of ozone with amino acids and proteins. *Atmospheric Environ* **3**: 669-82.

Mueller GA, Smith AM, Williams DC Jr, Hakkaart GA, Aalberse RC, Chapman MD, Rule GS, Benjamin DC (1997) Expression and secondary structure determination by NMR methods of the major house dust mite allergen Der p 2. *J Biol Chem* **272**: 26893-8.

Nelson HS, Hirsch R, Ohman JL, Platts-Mills TAE, Reed CE, Solomon WR (1988) Recommendations for the use of residential air-cleaning devices in the treatment of allergic respiratory diseases. *J Allergy Clin Immunol* **82**: 661-9.

Niven R McL, Fletcher AM, Pickering AC, Custovic A, Sivour JB, Preece AR, Oldham LA, Francis HC (1999) Attempting to control mite allergens with mechanical ventilation and dehumidification in British houses. *J Allergy Clin Immunol* **103**: 756-62.

Nogrady SG, Furnass SB (1983) Ionisers in the management of bronchial asthma. *Thorax* **38**: 919-22.

Ohman JL, Findlay SR, Leitermann SB (1984) Immunotherapy in cat-induced asthma: double-blind trial with examination of *in vivo* and *in vitro* responses. *J Allergy Clin Immunol* **74**: 230-9.

Olson JR, Klapper DG (1986) Two major human allergenic sites on ragweed pollen allergen antigen E identified by using monoclonal antibodies. *J Immunol* **136**: 2109-15.

Ono R, Oda T. Measurement of hydroxyl radicals in pulsed corona discharge. *J Electrostatics*, in press.

Osterballe O, Weeke B, Albrechtson O (1979) Influence of small atmospheric ions on the airways in patients with bronchial asthma. *Allergy* **34**: 187-94.

Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD (1994) Monoclonal antibodies to Group II *Dermatophagoides* spp. Allergens: Murine response, epitope analysis and development of a two-site ELISA. *J Allergy Clin Immunol* **94**: 537-46.

Palti Y, De Nour E, Abrahamov A (1966) The effect of atmospheric ions on the respiratory system of infants. *Pediatrics* **38**(3): 405-11.

Parametric Estimating Initiative (PEI) Parametric Estimating Handbook Second edition (2001), International Society of Parametric Analysts (ISPA). Web address: http://www.ispa-cost.org/PEIWeb/cover.html.

Paulo M, Gong P(1991) Respiratory effects of ozone: whom to protect, when and how? *J Respir Dis* **12**: 482-99.

Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, Woodcock AJ(1996) House dust mite allergens: a major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* **153**: 141-6.

Peden DB, Woodrow S, Devlin RB (1995) Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. *Am J Respir Crit Care Med* **151**: 336-45.

Pethig R (1983) The physical characteristics and control of air ions for biological studies. *J Bioelectricity* **2**: 15-35.

Platts-Mills TAE, Chapman MD (1987) Dust mites: immunology, allergic disease, and environmental control. *J Allergy Clin Immunol* **80**: 755-75.

Platts-Mills TAE, Heymann PW, Chapman MD, Hayden ML, Wilkins SR (1986) Cross-reacting and species specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for 'Antigen P_1 Equivalent' in house dust and dust mite extracts. *J Allergy Clin Immunol* **78**: 398-407.

Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD (1997) Indoor allergens and asthma: Third International Workshop, Cuenca Spain. *J Allergy Clin Immunol* **100**: S1-S24.

Podleski WK (1980) Asthma and ionisation. *Lancet* **ii**: 1035.

Rathborne J, Naylor S (1996). Postgraduate Research Projects, Asthma and Environmental Programme. Postgraduate Research Projects Annual Report. Oxford University Web Pages. Address: http://www.ecu.ox.ac.uk/annrep96/postgrad.html.

Reiser KM, Tyler WS, Hennessy SM, Dominquez JJ & Last JA (1987) Long-term consequences of exposure to ozone. II. Structural alterations in lung collagen of monkeys. *Toxicol Appl Pharmacol* **89**: 314-22.

Robinson C, Kalshekar NA, Srinvasan N, King CM, Garrod DR, Thompson PJ, Stewart GD (1997) On the significance of the enzymatic activity of mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterisation. *Clin & Exp Allergy* **27**: 10-21.

Sakaguchi M, Inouye S, Yasueda H, Irie T, Yoshizawa S, Shida T (1989) Measurement of allergens associated with dust mite allergy. *Int Arch Allergy Appl Immunol* **90**: 190-3.

Siebers RW, Patchett K, Crane J (1996) Mite allergen (Der p1) on children's clothing. *J Allergy Clin Immunol* **98**: 853-4.

Siebers RW, Rains N, Fitzharris P, Crane J (1998) House dust mite allergen (Der p 1) in human hair. *J Allergy Clin Immunol* **101**: 421-2.

Sigmond RS & Goldman M (1978) Corona discharge physics and applications. *NATO Advanced Study Inst Series B* **89**: 1-64.

Sigmond RS, Goldman M, Brenna D (1980) Corona corrosion of aluminium in air: electrochemical interaction between electrica coronas and metal surfaces. *Proc 6th Int Conf on Gas Discharges and their Applications (London)* pp82-5, IEE, London.

Smith AM, Chapman MD (1997) Localization of antigenic sites on Der p 2 using oligonucleotide-directed mutagenesis targeted to predicted surface residues. *Clin Exp Allergy* **27**: 593-9.

Smith JM, Disney ME, Williams JD, Goels ZA (1969) Clinical significance of skin reactions to mite extracts in children with asthma. *BMJ* **2**: 723-6.

Smith JJ, Olson JR, Klapper (1988) Monoclonal antibodies to denatured ragweed pollen *Amb a* I: characterization, specificity for the denatured allergen, and utilization for the isolation of immunogenic peptides of *Amb a* I. *Mol Immunol* **25**: 355-65.

Sporik RB, Holgate ST, Platts-Mills TAE, Cogswell J (1990) Exposure to house dust mite allergen (*Der p* I) and the development of asthma in childhood: A prospective study. *N Engl J Med* **323**: 502-7.

Squillace SP, Sporik RB, Rakes G, Couture N, Lawrence A, Merriam S, Zhang J, Platts-Mills TAE (1997) Sensitization to dust mites as a dominant risk factor for adolescent

asthma: multiple regression analysis of a population-based study. *Am J Respir Crit Care Med* **156**: 1760-4.

Stewart GA, Thompson PJ, Simpson RJ (1989) Protease antigen from house dust mite. *Lancet* **2**: 154-5.

Stryer L (1995) *Biochemistry*, (4th edition), WH Freeman, New York.

Sun B, Sato M, Clements JS (1997) Optical study of active species produced by a pulsed streamer corona discharge in water. *J Electrostatics* **39**: 189-202.

Taylor DM, Secker PE (1994) *Industrial Electrostatics: fundamentals and measurements,* Research Studies Press Ltd., Taunton, England.

Thompson SJ, Carswell F (1988) The major allergen of the house dust mite, *Dermatophagoides pteronyssinus* is synthesised and secreted into its alimentary canal. *Int Arch Allergy App Immun* **85**: 312-5.

Thomson JJ (1893) *Recent researches in electricity and magnetism.* Oxford University Press, London.

Tovey ER (1992) Allergen exposure and control. *Exp Appl Acarol* **16**: 181-202.

Tovey ER, Baldo BA (1990) Localisation of antigens and allergens in thin sections of house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pryoglyphidae). *J Med Entomol* **27**: 368-76.

Tovey ER, Chapman MD, Platts Mills TAE (1981) Mite faeces are a major source of house dust mite allergens. *Nature* **289**: 592-3.

Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE (1981) The distribution of dust mite allergen in the house of patients with asthma. *Am Rev Respir Dis* **124**: 630-5.

Tovey ER, Ford SA, Baldo BA (1989) Enhanced immunodetection of blotted house dust mite protein allergens on nitrocellulose following blocking with Tween 20. *Electrophoresis* **10**: 243-9.

Tovey ER, Marks GB, Mattews M, Green WF, Woolcock A (1992) Changes in mite allergen *Der p* I in house dust following spraying with a tannic acid/ acaracides solution. *Clin Exp Allergy* **22**: 67-74.

Trudinger M, Chua KY, Thomas WR (1991) cDNA encoding the major mite allergen *Der f* II. *Clin Exp Allergy* **21**: 33-7.

Van Metre TE Jr, Marsh DG, Adkinson NF Jr, Kagey-Sabotka A, Khattignavong A, Norman PS Jr, Rosenberg GL (1989) Immunotherapy decreases skin sensitivity to cat extract. *J Allergy Clin Immunol* **83**: 888-99.

Wahn U, Lau S, Bergmann R, Forster J, Bergman K, Bauer CP, Guggenmoos-Holzmann I (1997) Indoor allergen exposure is a risk factor for sensitisation during the first three years of life. *J Allergy Clin Immunol* **99**: 763-9.

Warner JA (1992) Environmental allergen exposure in homes and schools. *Clin Exp Allergy* **22**: 1044-5.

Weiss KB, Gergen PJ, Hodgson TA (1992). An economic evaluation of asthma in the United States. *N Engl J Med* **326**: 862-6.

Wickman M & Korsgaard J (1996) Transient sensitisation to house-dust mites: a study on the influence of mite exposure and sex. *Allergy* **51**: 511-3.

Wharton GW (1976). House dust mites. *J Med Ent* **12**: 577-621.

Witschi H (1988) Ozone, nitrogen dioxide and lung cancer; a review of some recent issues and problems. *Toxicol* **8**: 1-20.

Wood RA, Mudd KE, Eggleston PA (1992) The distribution of cat and dust mite allergens on wall surfaces. *J Allergy Clin Immunol* 89:126-30.

Woodcock A, Custovic A (1998) Role of the indoor environment in determining the severity of asthma. *Thorax* **53**: 547-51.

Yasueda H, Mia H, Yui Y, Shida T (1989) Comparative analysis of physicochemical and immunochemical properties of the two major allergens from *Dermatophagoides pteronyssinus* and the corresponding allergens from *Dermatophagoides farinae. Int Arch Allergy Appl Immunol* **88**: 402-7.

Yuuki T, Okumura Y, AndoT, Yamakawa H, Suko M, Haida M, Okudaira H (1991) Cloning and expression of cDNA coding for the major house dust mite allergen Der f II in Escherichia coli. *Agric Biol Chem* **55**: 1233-8.