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Journal of Electrostatics 60 (2004) 69–91
Journal of ELECTROSTATICS
www.elsevier.com/locate/elstat

The effect of corona discharge on dust mite and cat allergens

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Received 27 August 2003; accepted 30 November 2003

Abstract

Corona discharge has previously been reported to destroy the house dust mite allergen Der p1. This present paper describes the efficacy of corona discharge to destroy three more clinically important allergens and investigates the factors that affect this process. Using Der p1, Der p2, Der f1 and Fel d1 the allergen reducing efficacies of both negative and positive corona, of varying magnitudes, were assessed. The effect of the duration of exposure, initial allergen concentration and ozone, a by-product of corona discharge, was also investigated. The time course of allergen reduction in situ was also followed using an experimental and a commercially available, ionizer. Negative corona was found to be the most efficacious polarity in the majority of cases. Fel d1 reacted the least with positive corona products, which may be due to the primary structure of this allergen. The in situ tests showed that it was possible to destroy surface allergen up to 4 m away from the corona source, but this reduction was not found to be time-dependent over the three week exposure period. Further research directed at improving existing ionizer designs and the development of protocols for the safe use of ionizers to destroy domestic allergen reservoirs would be extremely beneficial.

Keywords: Corona discharge; Ionizers; Der p1; Der f1; Der p2; Fel d1; Allergens; Ozone; Domestic; Asthma

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doi:10.1016/j.elstat.2003.11.005

1. Introduction

Methods of reducing exposure to allergens are important in the avoidance and control of atopic asthma. Many different attempts to eradicate house dust mites (HDMs) and pet allergens from the domestic environment have been tried with varying levels of success. The use of ionizers have been investigated for benefits to asthma sufferers and has focused on the direct action of the ions themselves on human physiology [1,2], together with the filtration and precipitation of airborne dust and allergens [3]. The results of these tests have been ambiguous and contradictory and at present no conclusive clinical benefit has been demonstrated. Their use in the domestic environment to alleviate, or prevent, the symptoms of asthma and allergies is therefore not recommended at the present moment [4].

Recent studies have shown the possibility of a new, revised application for ionizers in asthma prevention. It has previously been reported that the products of corona discharge, the process by which ionizers produce ions, can destroy the major European HDM (*Dermatophagoides pteronyssinus*) allergen Der p1 [5]. This was shown using a simple pin-to-plane electrode arrangement as described later in this paper. It has also been shown that the corona products from commercially available ionizers are capable of destroying this allergen at a distance of 4 m away from the corona source [6]. It may therefore be possible to use ionizers in the domestic environment to reduce the allergenic load.

The occurrence of corona discharge in the high-intensity electric field region around the point electrode in electrodes of a non-uniform geometry, results in the production of monovalent ions of the same polarity as the point. A number of different ions can be created during corona discharge depending on the composition of the atmosphere surrounding the point electrode. These ions are then attracted to the opposite polarity electrode and drift towards the plane [7]. Chemically potent atomic and molecular radicals known as neutral metastable species are also produced in the interelectrode space [8].

The characteristics of this corona discharge differ depending upon the magnitude and polarity of the high voltage applied to the electrode. For negative corona in air, current pulsations are first to appear. These phenomena are known as *Trichel pulses* and, if the applied voltage is increased, these pulsations are dampened and eventually produce the *continuous glow* corona [9]. For positive coronas, the burst pulses will either join, becoming a regularly pulsating *positive glow* covering the active electrode, or the largest will develop into *positive streamers*. A number of ions and other corona products are produced in the pulse regimes of both polarities [10–12], and a greater variety of ions are produced in the higher current regimes [13].

In this present paper, we describe several properties of the corona-destruction of allergens. The efficacies of the two corona polarities at destroying previously untested common allergenic proteins were studied and the effect of varying exposure times and the magnitude and polarity of the corona current, spanning both the pulse and continuous glow regimes, were also examined. The allergens tested were the major European house dust mite (*Dermatophagoides pteronyssinus*) allergens Der p1 and Der p2, the major American house dust mite (*Dermatophagoides farinae*)

allergen Der f1, and the major domestic cat (*Felus domesticus*) allergen Fel d1. With knowledge of the optimal corona current, polarity and exposure time for destroying allergens, improved ionizers may be built, designed specifically for reducing the level of both aeroallergens and the allergenic reservoir of the domestic environment.

Molecular ozone, a powerful oxidizing corona product, was also investigated to determine whether this was responsible for the effects of corona discharge on allergens. Ozone is known to react with the amino acid residues of proteins in solution [14] but the effect of this oxidizing agent on dry, evaporated samples of proteins, which more closely mimics natural environmental conditions, have not previously been investigated.

With the possibility of using ionizers in situ, and also the prospect of developing improved ionizers for this use, it is important to determine the effects of corona products on allergens present in differing initial concentrations. Other denaturant products used to destroy allergens in situ, such as tannic acid, have been found to have differing efficacies depending on the amount of baseline allergen present [15]. Medical specialists have therefore recommended that such products be investigated and state their effectiveness at destroying allergens on the basis of the concentration of allergens before treatment. In order to achieve this for a corona-based treatment, the efficacy of corona discharge to destroy clinically important allergens, as described above, were tested with differing initial concentrations. It was then possible to determine whether the amount of allergen present affected the percentage reduction in concentration obtained, and also the number of allergen molecules destroyed, per unit exposure.

The time course of the allergen-destroying efficacy of an experimental nine-pin ion wind generator and a commercially available, long wire-to-electrode ionizer was also followed in order to determine an optimal period of exposure for in situ applications. These ionizers were placed in an unoccupied, furnished room and the pattern and extent of any corona-destruction of the allergen was then compared and discussed in relation to the relative positions of the samples to the ionizer, the ionizer specifications and any influence the furniture may have had.

2. Methods

2.1. Preparation of evaporated aqueous allergen samples

Evaporated allergen samples were prepared as previously described [16,5] and used throughout because these are inexpensive, have high concentration homogeneity and allow detailed investigation into the effects of corona discharge without the problems of powder charging and associated sample loss. Differing masses of dust, or HDM culture, were used to produce the aqueous solutions with a concentration of the desired allergen at approximately 100 ng ml^{-1}.

Solutions of Der p1 and Der p2 were made using a HDM culture, cultivated from material obtained from British domestic vacuum cleaner bags. Der f1 was prepared using material obtained from American domestic vacuum cleaner bags. A Fel d1

solution was prepared using material obtained from British vacuum cleaner bags. 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% as a preservative. Evaporated samples of this solution were then prepared by pippetting 100 μl of the desired allergen solution onto aluminum foil. The sample and its paired control were prepared adjacently. These were then dried at 37°C.

2.2. Investigation into different variables of corona exposure

A simple pin-to-plane corona electrode arrangement was made with a stainless steel pin (point radius 45 μm) inserted through the top of a Perspex frame. The lower surface of the frame, 15 mm below the pin was covered with a thin sheet of aluminum metal to form the planar ground electrode.

2.2.1. Exposure time

An evaporated allergen sample was fixed to the planar electrode and the pin was connected to a DC high voltage supply (Model 3807: Alpha Series III, Brandenberg Ltd., Surrey, UK). The allergen samples were exposed for 1, 15, 30, 45, 60, 120, 240 and 300 min. The samples were then removed and stored for subsequent assay by the appropriate two-site monoclonal antibody ELISAs (INDOOR Biotechnologies Ltd., Cardiff, UK). See Table 1 for more details on the antibodies used in the ELISA tests. For negative corona, ionic bombardment equivalent to a corona current of 25 μA was used. For positive corona this current was 5 μA. These values were chosen as being representative of the desired type of corona (Trichel, or pulse) generated at a constant relative humidity of 45%. The corona current was maintained at a constant value throughout each test. 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 corona polarity.

2.2.2. Corona polarity and magnitude

The allergen sample was exposed for 60 min to corona discharge at different corona currents. Corona currents of $2, 5, 10, 25, 30, 40, 50, 80, 90$ and $100\ \mu A$ were maintained with the negative polarity and positive corona currents of

Table 1
The antibodies utilised in the various two-site monoclonal antibody ELISA kits to detect the allergens under investigation

Allergen	ELISA Kit	Capture antibody	Biotinylated antibody	Reference
Der p1	Der p1	α-Der p1, 5H8	α-Group 1, 4C1	[17]
Der f1	Der f1	α-Der f1, 6A8	α-Group 1, 4C1	[17]
Fel d1	Fel d1	α-Fel d1, 6F9	3E4	[18]
Der p2	Group 2	α-Group 2, ID8	7A1	[19]

2, 5, 10, 15, 20, 25 and 30 μA were also investigated. The positive currents only ranged from 2 to 30 μA due to the current/voltage characteristics of corona discharge in air (for a review see, Ref. [9]) with the pin-to-plane electrode spacing used. The transition points where pulse turned to continuous glow corona were 65 and 17 μA for negative and positive corona, respectively. Ten replicates were completed for each corona current and polarity.

2.2.3. Initial allergen concentration

The allergen solutions were diluted using distilled water to produce concentrations ranging from 40 to 1800 ng ml^{-1} and the samples then prepared as described above. The allergen samples were fixed, in turn, onto the earthed, planar electrode of the corona apparatus and exposed to negative Trichel corona with a corona current of 25 μA for 120 min. The sample was then removed and stored for subsequent assay. The control allergen sample was exposed to the ambient atmosphere for each test period, and then also stored for later assessment. 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 min per sample.

Data from this set of experiments was used to calculate the amount of allergen destroyed, in moles, by using the values of the molecular mass for each allergen (Der p1, 24 kDa; Der f1, 24 kDa; Der p2, 14 kDa; Fel d1, 36 kDa) and the concentration data obtained from the results of the ELISA. One mole of a compound has a mass equal to its relative molecular mass expressed in grams. The number of moles of allergen destroyed was 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).

2.2.4. Exposure to molecular ozone

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 at a constant rate through an outlet tube (10 mm diameter), and onto the allergen sample 10 mm below the aperture. The ozone concentration, measured using short-term ozone detection tubes (Dräger, 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 one pin were approximately 0.5–1 ppm. A higher concentration of 50 ppm 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 2 l min^{-1} and applying 170 V to the ozone-generating unit.

Following a period of 5 min, in which the concentration of ozone was allowed to stabilize, the foil with the allergen sample was fixed below the outlet tube and exposed for 1 h. After this time, 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 f1, Der p2 and Fel d1. Eight replicates were completed for each allergen.

2.3. In situ tests

2.3.1. Construction of the nine-pin experimental ion wind generator

The experimental ion wind generator was constructed by soldering nine pins to a vertical grille of 10 mm^2 mesh. This was then fixed into a plastic base 30 mm from a similar grille connected to earth as illustrated in Fig. 1. The grille with the point electrodes could then be connected to the negative DC, 15 kV high voltage generator.

2.3.2. Measurement of ion production and ion wind velocity

Fig. 2 illustrates the apparatus used to measure the rate of ion production. The ionizer under test was placed into an electrically grounded chamber onto a 1 m^2 sheet of Perspex (10 mm thick) and connected to its power supply. An aluminium foil-covered frame was then placed over the ionizer to act as the ion collector and then connected to an electrometer (Model: 610C, Solid state electrometer, Keithley Instruments, OH, USA). The ionizer was then switched on and the resultant current flowing through the frame measured. The rate of monovalent ion production could 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). Dividing the value of Q with the charge of an electron (1.66×10^{-27} C) thus gave the number of negative, monovalent ions produced by the ionizers tested in situ. The velocity of the ion wind was measured using a combination air velocity and flow meter (Model: AV2; Airflow Developments Ltd., Buckinghamshire, England).

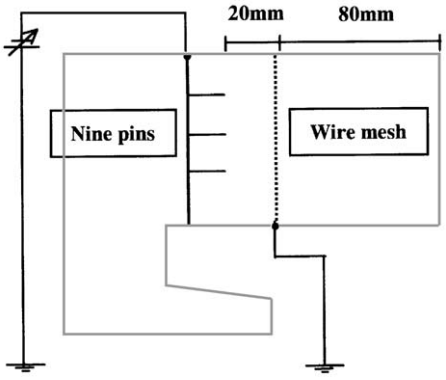

Fig. 1. Schematic diagram of the experimental nine-pin ion wind generator.

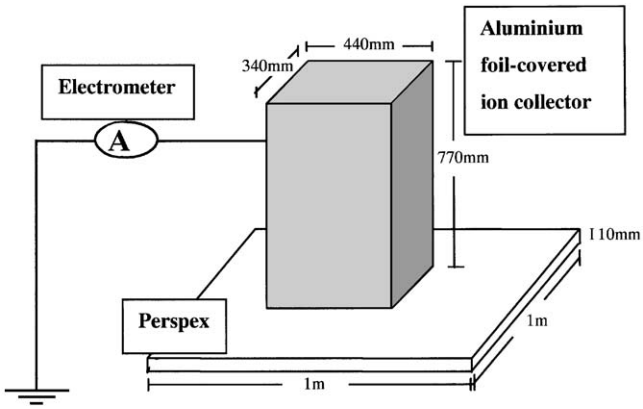

Fig. 2. Diagram of the apparatus used to measure the total rate of ion production from the experimental and commercial ionizers. This apparatus was placed inside an electrically grounded chamber to screen from background interference. The ionizer was placed inside the ion collector and connected to its power supply. The resultant current flow through the ion collector was measured using the electrometer.

2.3.3. Room-scale exposure of Der p1 samples to the nine-pin ion wind generator and the Ionic Breeze™ Silent Air Purifier

Twelve groups of six evaporated Der p1 samples were placed in an unoccupied, furnished, office room measuring $2.9 \times 6.8 \times 2.93$ m (see Fig. 3). 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 (0.90 m above the floor). The samples were positioned in radial sectors equidistant from the ionizer at 0.3, 1.2, 2.4 and 4 m for Sectors 1, 2, 3 and 4, respectively. Samples 2.2 and 4.2 were placed on the floor 1.05 m below the center of the ion wind generator and Samples 3.1 and 3.3 were fixed vertically against the walls.

The ion wind generator was connected to a negative DC, 15 kV power supply for 1,2 or 3 weeks. Sixteen control samples were kept in identical conditions without the ion wind generator present. The door was briefly opened every 2 days to measure the ozone concentration in the room.

The above protocol was repeated 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*™ ionizer was connected to a 110 V mains transformer for 1, 2 or 3 weeks and Der p1 samples were placed in position. The door was briefly opened every 2 days to measure the ozone concentration and to clean the planar electrodes of the ionizer with a dry cloth in accordance with the manufacturer's instructions. After the duration of exposure the samples and controls were prepared for ELISA.

2.4. Data analysis

For each corona exposure, the allergen concentrations of each sample was compared to its paired control by the F-test. The concentration data from each

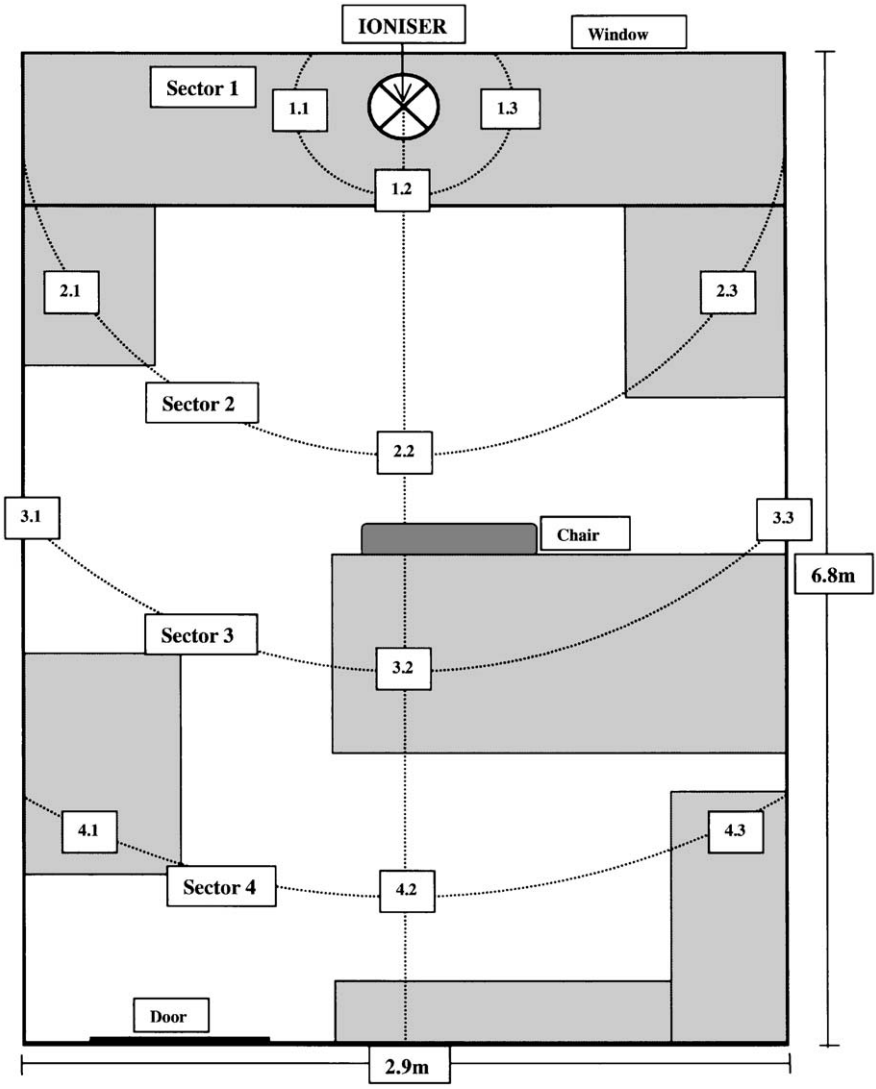

Fig. 3. Map of sample positions in the unoccupied office room for in situ tests of the experimental and commercial ionizers. Three sets of six samples were arranged in radial sectors equidistant from the ionizer (Sector 1 = 0.30 m, Sector 2 = 1.20 m, Sector 3 = 2.40 m, Sector 4 = 4.00 m). The tables were of approximately the same height (0.90 m), and the chair, in line with the ionizer, was 0.1 m taller. Samples 2.2 and 4.2 were placed on the floor 1.05 m below the level of the ionizer and Samples 3.1 and 3.3 were fixed vertically to the walls.

experimental group was then analyzed using the Paired *t*-test for means, or the Mann–Whitney-*U* test where appropriate. These data were then presented as mean percentage reductions in allergen concentration, calculated by comparing the allergen sample to its equivalent control in the specific time period.

The Mann–Whitney-U test was also used to compare the percentage reductions caused by either the different corona currents and polarities and also to compare the percentage reduction data between sample positions over the different exposure times in the in situ tests. Spearman Rank correlations were calculated from the percentage reduction data and the number of destroyed moles of allergen against the initial allergen concentration. The Kruskal–Wallis non-parametric ANOVA was used on the number of destroyed moles of allergen values to determine whether a difference between the highest and lowest median existed in the sample groups. This latter test was also used to determine whether the percentage reductions in allergen concentration, caused by different exposure times, were statistically different from each other. Statistical significance was defined as $P<0.05$.

3. Results

3.1. The effect of different periods of exposure to either negative or positive corona discharge

Negative corona discharge has been found to destroy all allergens tested in a time-dependent manner. The concentration of the control samples in all tests were relatively constant, therefore the percentage reductions can be validly calculated and used to compare the results between the different allergens.

The values of percentage reduction in Der p1 concentration are different from those previously reported [5] because of the different initial concentrations used. Those values presented earlier were from an initial concentration of 344.69 ± 15.66 ng ml^{-1} ($n=53$) and reached a maximum reduction of 50.50% after 300 min with a concentration of 285 ng ml^{-1}. The initial concentration of all allergens in this report however was kept constant at approximately 100 ng ml^{-1} to enable easier comparison with the other allergens under investigation. This is an important variable that is discussed in more detail in Section 3.6 and is an important factor to attend to when attempting to replicate results. Fig. 4A shows the strong, positive correlation between the percentage of Der p1 destroyed and the period of exposure (rho $=0.896$, $p<0.01$). Reductions were observed that ranged from the insignificant $0.49\pm1.77\%$ after 1 min to $59.58\pm10.17\%$ after 300 min. All reductions were significant after 30 min.

Fig. 4B shows the reductions in Der f1 concentration observed after exposure to negative corona ranging from $3.55\pm4.83\%$ after 1 min to $96.70\pm1.45\%$ after 300 min. All reductions were highly significant after 45 min of exposure or more ($P<0.01$), correlated well with the period of exposure (rho $=0.896$, $P<0.01$) and fit the exponential growth to maximum trend line well ($R^2=0.985, 0.960$). Exposure to positive corona caused reductions that ranged from $1.23\pm2.82\%$ after 1 min, to $48.34\pm11.12\%$ after 300 min (rho $=0.776$, $P<0.01$; $R^2=0.960$).

Fig. 4C shows the reductions in Der p2 concentration after exposure to negative corona, ranging from $2.33\pm2.17\%$ after 1 min to $96.57\pm1.63\%$ after 300 min (rho $=0.921$, $P<0.01$; $R^2=0.948$). The reductions observed after positive corona

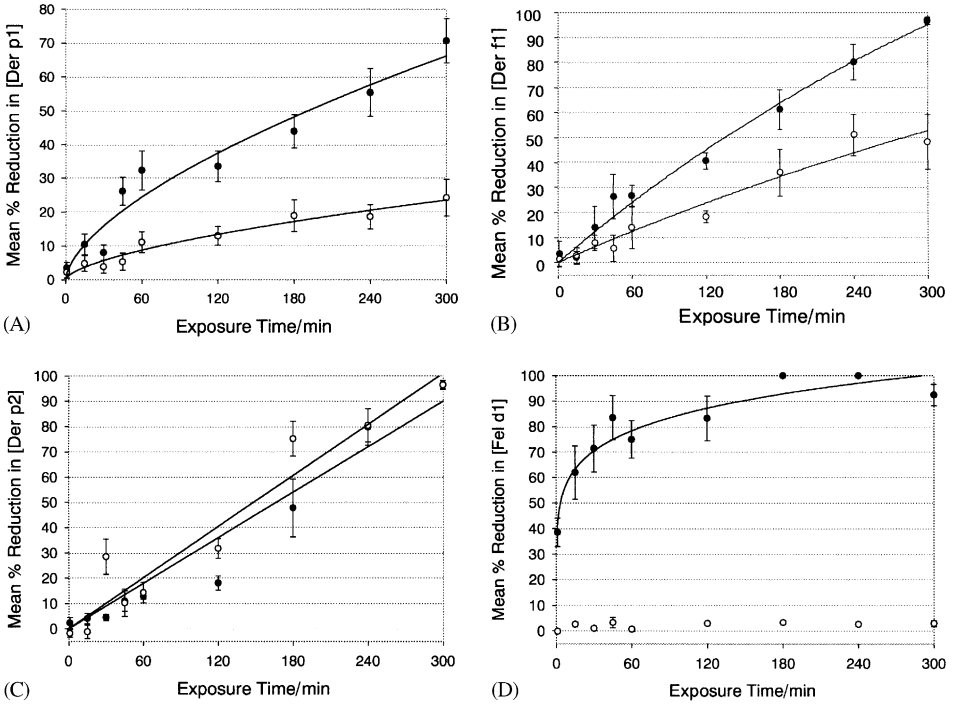

Fig. 4. The percentage reduction in (A) Der p1, (B) Der f1, (C) Der p2 or (D) Fel d1 concentration after exposure to negative (●), or positive (◦) corona ($n = 6$, SEM shown).

treatment also fit the exponential growth to maximum curve well ($R^2 = 0.933$) with reductions that ranged from $-1.79 \pm 1.48\%$ after 1 min, to $96.42 \pm 1.69\%$ after 300 min (rho = 0.921, $P < 0.01$). All reductions were significant after 30 min of exposure or more ($P < 0.05$).

Unlike the trends observed in all other allergens tested, the percentage reductions in Der p2 concentration were very similar after exposure to negative or positive corona discharge. Only those reductions caused after 30 and 120 min were statistically different from each other ($P < 0.05$).

The allergen concentrations of the *Dermatophagoides* mite experienced statistically similar reductions after exposure to negative corona. After exposure to positive corona, all *Dermatophagoides* allergens also experienced similar reductions except after an exposure period of 120 min or more ($P < 0.05$). After this time all *Dermatophagoides* allergens experienced statistically similar reductions except after an exposure period of 120 min 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. This exception warrants further research and may shed light on possible structural, or chemical differences between this allergen and the *Dermatophagoides* allergens.

After exposure to negative corona, reductions in Fel d1 sample concentrations were observed that ranged from $38.54 \pm 5.53\%$ after 1 min to $100.00 \pm 0.00\%$ after 180 and 240 min (Fig. 4D). All reductions were highly significant ($P < 0.01$) and fit the trend line well (rho = 0.672, $P < 0.01$; $R^2 = 0.924$). The seemingly anomalous result after 300 min of exposure, which was only reduced by $92.45 \pm 4.19\%$, is probably due to statistical variation.

Unlike the *Dermatophagoides* allergens, exposure to positive corona caused very little reduction in the samples' Fel d1 concentration. The percentage reductions observed ranged from $-0.18 \pm 0.15\%$ after 1 min, to $3.27 \pm 0.36\%$ after 180 min. Only the reductions after 120 min 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 ($P = 0.097$). The overall mean reduction caused by positive corona was $2.11 \pm 0.35\%$ ($P < 0.01$, $n = 54$).

3.2. The effect of molecular ozone

No statistically significant differences in allergen concentration occurred after exposure of the evaporated samples of all allergens to 50 ppm ozone for 60 min. All allergen concentrations remained at approximately 100 ng ml^{-1}.

3.3. The effect of differing magnitudes of negative and positive corona

Table 2 shows the mean concentration of allergens in the samples exposed to negative corona at different corona currents. Allergen samples were significantly

Table 2
The mean allergen concentrations in samples (Spl) exposed to negative corona discharge at different corona currents and their paired controls (Con)

Corona current	Mean [Allergen]/(ng ml^{-1})							
(μA)	Der p1		Der f1		Der p2		Fel d1	
	Con	Spl	Con	Spl	Con	Spl	Con	Spl
2	105.09	97.70	97.91	92.02	97.14	95.17	100.47	79.44
5	98.84	82.54	106.76	87.66	105.78	98.89	102.36	67.88
10	102.49	78.19	100.43	77.00	105.06	91.12	106.25	45.94
25	101.93	71.16	101.64	73.98	101.03	85.32	103.19	26.92
30	100.89	71.04	106.80	77.18	100.36	83.88	106.59	21.99
40	103.14	70.93	97.39	69.05	107.53	92.31	106.63	23.10
50	103.09	69.43	101.91	76.15	104.96	87.65	100.63	28.41
80	105.90	70.69	103.17	76.77	107.51	90.77	103.12	24.07
90	103.39	64.77	101.50	75.47	111.59	95.61	104.44	26.93
100	102.90	65.17	99.27	70.03	104.13	87.80	100.51	23.52

Each value is the mean of 10 replicates; controls were exposed to the ambient atmosphere with no corona products present.

lower than their paired controls after exposure to negative corona at all currents, or at positive currents of 5 μA or above.

Fig. 5 shows that the mean percentage reductions in all allergen concentrations followed an exponential growth to maximum relationship with respect to the magnitude of corona current. Fel d1 was affected the most with reductions that ranged from $20.65 \pm 6.93\%$ at 2 μA, to $79.08 \pm 3.28\%$ at 30 μA (rho = 0.551, $P < 0.01$; $R^2 = 0.945$). Der p1 and Der f1 samples exhibited statistically similar reductions in concentration ($P > 0.05$). Reductions in Der p1 concentration ranged from $6.91 \pm 2.26\%$ at 2 μA, to $36.72 \pm 7.45\%$ at 100 μA (rho = 0.408, $P < 0.01$; $R^2 = 0.954$). Reductions in Der f1 ranged from $6.10 \pm 2.09\%$ at 2 μA, to $29.60 \pm 6.45\%$ at 100 μA (rho = 0.311, $P < 0.01$; $R^2 = 0.945$). Reductions in Der p2 concentration ranged from the insignificant $2.02 \pm 1.91\%$ at 2 μA, to $16.12 \pm 4.39\%$ at 50 μA (rho = 0.302, $P < 0.01$; $R^2 = 0.942$).

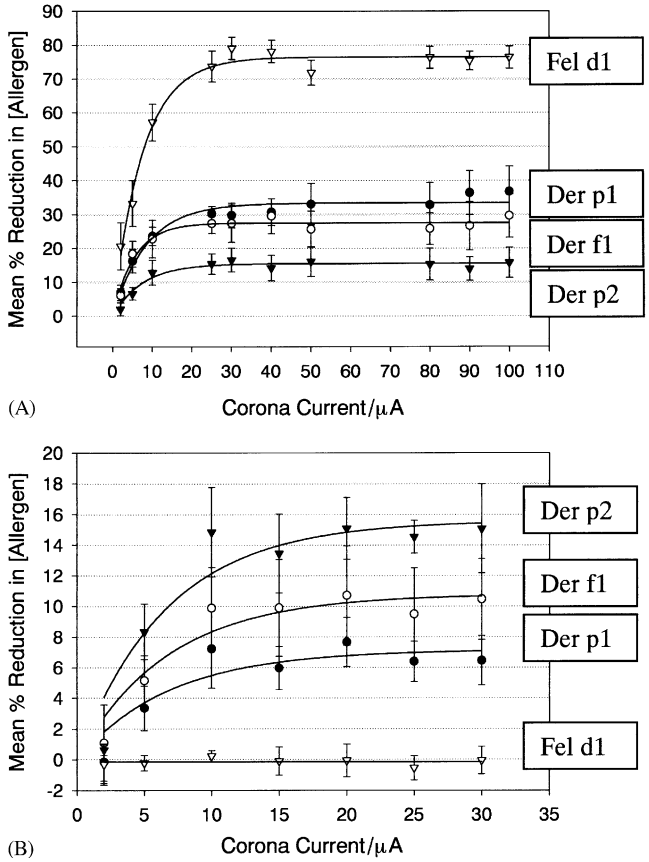

Fig. 5. The percentage reductions in Der p1 (●), Der f1 (◦), Der p2 (▼) and Fel d1 (▽) allergen concentration after exposure to either (A) negative, or (B) positive corona with different corona currents ($n = 10$, SEM shown).

Table 3
The mean allergen concentrations in samples (Spl) exposed to positive corona discharge at different corona currents and their paired controls (Con)

Corona current	Mean [Allergen]/(ng ml^{-1})							
(μA)	Der p1		Der f1		Der p2		Fel d1	
	Con	Spl	Con	Spl	Con	Spl	Con	Spl
2	104.32	104.46	101.61	100.48	102.77	102.11	99.55	99.90
5	98.97	95.58	100.81	95.55	101.64	93.10	100.52	100.68
10	100.11	92.80	104.46	94.23	99.24	84.83	103.35	103.07
15	101.99	95.70	102.91	92.71	102.77	88.71	101.13	101.12
20	104.79	96.77	97.41	87.05	101.70	86.17	100.31	100.34
25	101.21	94.71	104.33	94.59	99.63	85.08	102.48	103.05
30	103.45	96.62	102.32	91.72	98.83	83.92	98.84	98.86

Each value is the mean of 10 replicates.

Table 3 shows the mean concentrations in the allergen samples exposed to positive corona discharge at different corona currents and their paired controls. All *Dermatophagoides* allergens were significantly lower in concentration than their paired controls after exposure to positive corona at 5 μA of current or more ($P<0.05$). However, no Fel d1 samples were significantly reduced after exposure to any positive corona current for the exposure period of 60 min.

Der p2 samples exhibited the greatest loss in concentration, as shown in Fig. 3 and are statistically similar to the reductions observed after negative corona. This pattern can also be seen in Section 3.1 with a corona current of 25 μA. The efficacy of corona to destroy this allergen is the same regardless of the polarity. These reductions ranged from $0.64\pm0.36\%$ at 2 μA, to $15.09\pm2.01\%$ at 20 μA (rho = 0.518, $P<0.01$; $R^2 = 0.887$). Reductions in Der f1 concentration were found to range from $1.09\pm2.47\%$ at 2 μA, to $10.71\pm3.24\%$ at 20 μA (rho = 0.302, $P<0.01$; $R^2 = 0.913$). Small reductions in Der p1 concentration were also observed that ranged from $-0.14\pm1.38\%$ at 2 μA, to $7.68\pm1.61\%$ at 20 μA (rho = 0.380, $P<0.01$; $R^2 = 0.815$).

Again, the statistically similar reductions in Der p1 and Der f1 concentrations, at each current value of either polarities, are probably due to Der p1's high (81%) primary sequence homology to Der f1 [20–22] and the stability of Fel d1 during positive corona may also be due to the allergen's primary structure.

3.4. The effect of differing initial allergen concentrations

All allergens tested showed similar behavior upon irradiation with corona discharge (see Figs. 6 and 7). 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 h exposure with a constant corona current, however, remained relatively constant. Only Der p1 showed a significant positive correlation between the initial concentration and the

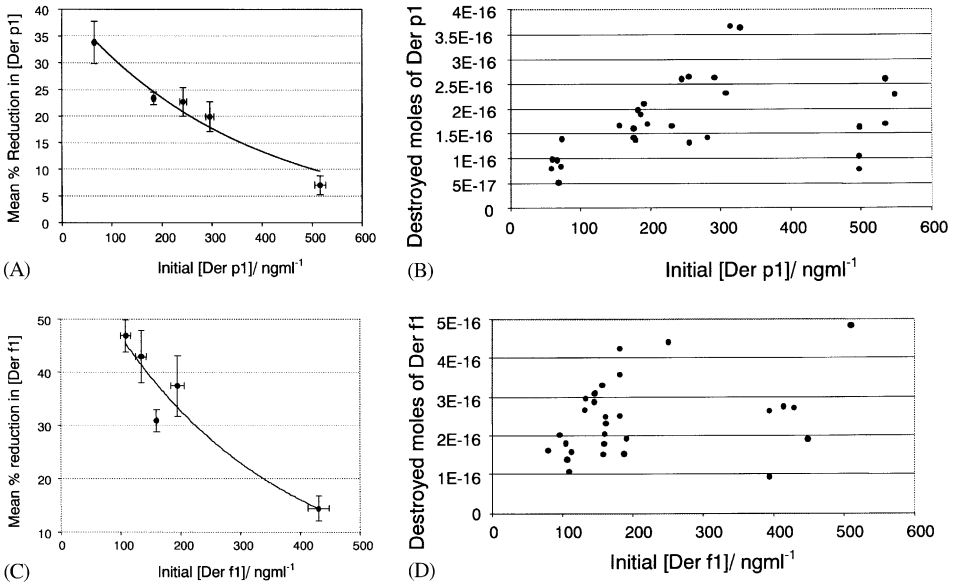

Fig. 6. The relationship between the initial concentration of either Der p1 or Der f1 in the sample and both the mean percentage reduction in allergen concentration observed ($n = 6$, SEM shown) and the number of destroyed moles of allergen after 120 min of negative corona.

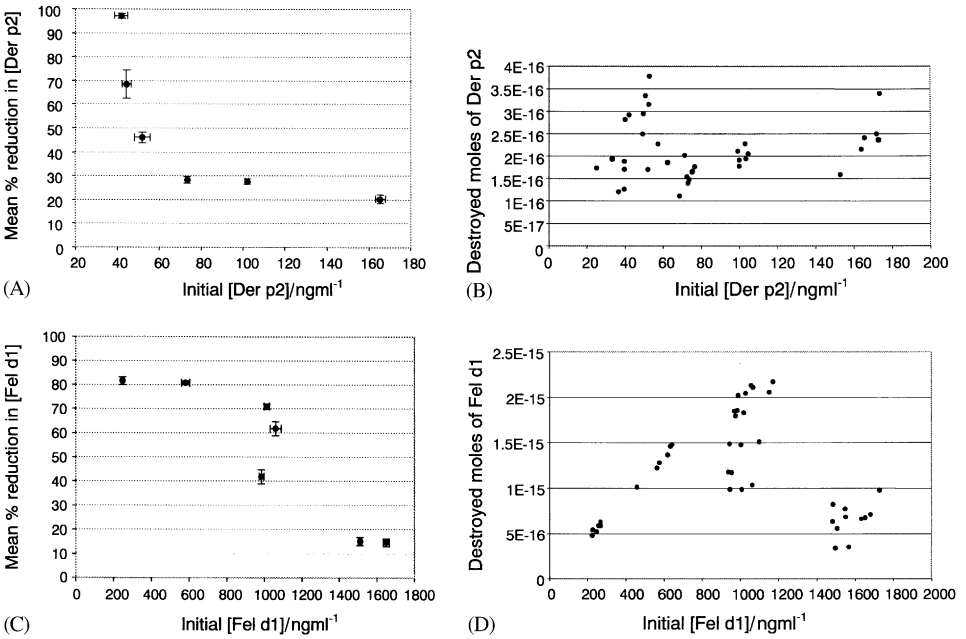

Fig. 7. The relationship between the initial concentration of either Der p2 or Fel d1 in the sample and both the mean percentage reduction in allergen concentration observed ($n = 6$, SEM shown) and the number of destroyed moles of allergen after 120 min of negative corona.

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 (using the Kruskal–Wallis, non-parametric ANOVA), all four allergens had similar mean values for the number of moles of allergen destroyed. These are presented in Table 3. 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 h exposure to negative corona discharge with a corona current of 25 μA ($n = 138$).

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 and therefore the fraction of destroyed molecules were smaller when the initial concentration was greater. This stochiometric relationship between the active corona product(s) and the amount of allergen destroyed thus has bearing on its potential use in situ. The amount of allergen destroyed per unit exposure will be the same regardless of the concentration present before treatment, unlike other methods of allergen destruction reported in the literature [15].

3.5. Room-scale exposure to the nine-pin experimental ionizer

The effect of the experimental and commercially available ionizer has previously been reported for a 1 week exposure [6]. Those tests used similar initial concentrations to those used in the exposures reported here (227.65 ± 6.96 ng ml^{-1}) and caused statistically significant reductions in the Der p1 concentration of the samples. 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 1, 2 or 3 weeks in the unoccupied, furnished office room. 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 ionizer. The control concentrations were relatively constant with mean concentrations of, 225.27 ± 4.24 and 228.33 ± 2.94 ng ml^{-1} for the 1, 2 and 3 week exposures, respectively ($n = 16$). The ozone concentration in the room, measured at a distance of 6 m from the ion wind generator, was a constant 0.05 ppm throughout the 1, 2 and 3 week exposures (Table 4).

The majority of observed reductions in Der p1 after 2 weeks of exposure, shown in Fig. 8B, were similar in magnitude to the samples exposed for 1 week. 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 1 week in the equivalent positions ($P<0.05$). It is unclear why these reductions were lower after 2 weeks of exposure although the general patterns noted for the reductions after 1 week also appear in the samples exposed for 2 weeks. That is, the reductions in samples placed directly in line with the ionizer were always statistically lower than those placed on either side when compared using the Mann–Whitney U test ($P<0.05$) suggesting that the ion wind forms a divergent plume or bifurcates upon exiting the ionizer thus leaving a lower concentration of corona products in the middle.

Table 4
The mean number of moles destroyed for each allergen investigated and the overall mean number of moles destroyed

Allergen	Mean number of models 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}

Another explanation for the lower reductions in the samples positioned in line with the ionizer could be due to their relative positions in the room: Samples 1.2 were placed 0.3 m in front of the ionizer but below the flow of the ion wind; Samples 2.2 were placed on the floor 1.05 m below the center of the ion wind generator; Samples 4.2 were also placed on the floor but with the desk and chair in line with the ionizer. These sample positions, particularly those fixed to the floor, could receive less exposure to the corona products than those 0.9 m above the floor and fixed horizontally (i.e., Samples 1.1, 1.3, 2.1, 2.3, 4.1 and 4.3).

The positions of samples 3.1 and 3.3 were fixed vertically to the walls 0.9 m above the floor and 2.4 m away from the ionizer. The lower reductions observed in these positions may 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 0.9 m above the floor on the desk, was positioned with a soft-furnished office chair, with a height of 1 m, in between the desk and the ionizer. The chair might have shielded the samples from the corona products.

The reductions observed after three weeks (Fig. 8C) show a different pattern of reductions. Although a time-dependent increase in reductions was not observed, even after 3 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. A possible reason why the reductions in Sample 1.2 were greater than in previous exposures ($P<0.05$) may be linked to the absence of the pattern noted above. After 3 weeks of exposure, it is possible that the diffusion of corona products enabled the samples on the floor in Sector 4, and just below the ionizer in Sector 1, to receive approximately the same quantity of active corona product(s) as those samples positioned to the sides of the room.

3.6. *Room-scale exposure of samples to the Ionic Breeze™ Silent Air Purifier*

The commercially available *Ionic Breeze™ Silent Air Purifier* ionizer had a higher rate of ion production of 1.904×10^{15} ions per second. The velocity of the ion wind

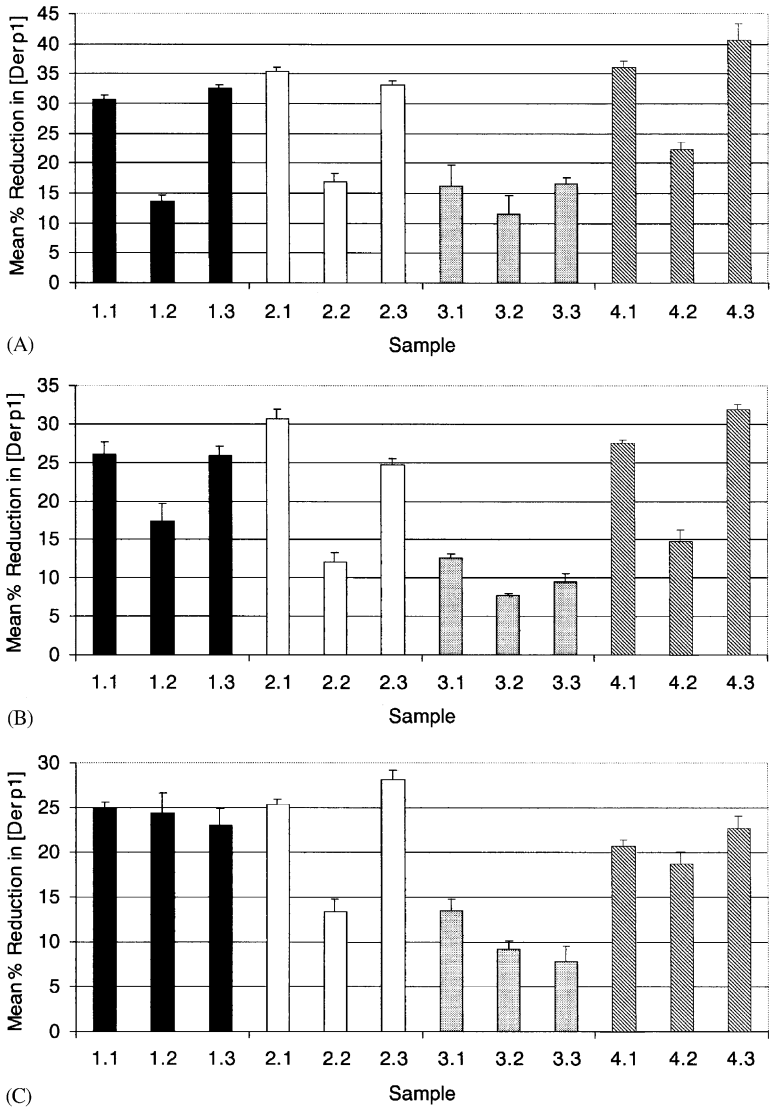

Fig. 8. The mean percentage reductions in Der p1 concentration of samples in various positions around the furnished room after a (A) 1 week exposure, (B) 2 week exposure, or (C) 3 week exposure to the experimental ion wind generator. The sample numbers refer to the position in each sector as shown in Fig. 3. All values are statistically significant ($n = 6$; SEM shown).

differed depending upon which side of the ionizer 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.

The results of this test showed that exposure to the corona products of the *Ionic Breeze*^{TM} *Silent Air Purifier* in the furnished office room led to statistically significant

reductions in the Der p1 concentration of the samples. The control concentrations were relatively constant with mean concentrations of 222.54 ± 3.41 and 225.33 ± 4.17 ng m1^{-1} for 2 and 3 week exposures, respectively ($n = 16$). These are comparable to the previously reported exposure for 1 week (223.35 ± 3.56 ng ml^{-1}) [6]. All samples, except Sample 4.2 after 2 week exposure and Samples 4.1, 4.2 and 4.3 after 3 week exposure, were statistically lower than their controls ($P < 0.05$). The ozone concentration in the room, throughout the three exposure periods, was negligible, although a concentration of 0.025 ppm was recorded immediately in front of the ionizer.

Fig. 9 shows the mean percentage reductions in Der p1 concentration of the samples after exposure to the *Ionic Breeze*^{TM} ionizer. After 2 weeks exposure (Fig. 9B) the percentage reductions ranged from $25.64 \pm 4.36\%$ in Sample 3.2, to the non-significant $5.31 \pm 2.44\%$ reduction in Sample 4.2. As found in the tests with the experimental ionizer, the majority of the samples' reductions observed after 2 weeks exposure were similar in magnitude to those achieved after 1 week of exposure. The large reduction in Sample 3.2 could be explained by the height of the *Ionic Breeze*^{TM} ionizer, which was 338 mm (electrode length) taller than the experimental ionizer. This would have enabled the corona products to overcome the shielding effect of the soft-furnished chair in line with the ionizer and Sample 3.2 and so destroy more Der p1 than the corona produced with the nine-pin experimental ionizer.

After 3 weeks exposure to the *Ionic Breeze*^{TM} ionizer, the reductions observed in the office room (see Fig. 9C) ranged from $20.72 \pm 4.44\%$ in Sample 1.2 to the non-significant $1.03 \pm 0.86\%$ in Sample 4.1. The reductions achieved after this 3 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 1 and 2 week exposures ($P < 0.05$), and the reductions in Sample position 3.3 were only greater than 1 week exposure in the same position ($P < 0.05$).

Unexpectedly, the samples in Sector 4 were not significantly reduced in this 3 week exposure to the commercial ionizer. Except for the reductions in Sample 4.3 after 2 weeks exposure and Sample 4.1 after 1 week of exposure, very little Der p1 was destroyed in samples in this sector 4 m from the ionizer. This is the only indication that the Der p1-destroying efficacy of the commercial ionizer decreases with an increase in distance from the ionizer. This could be due to the greater diffusion of the ion wind with the commercial ionizer than the experimental ionizer; the more diffuse corona products may be captured by the electrically earthed walls and furnishings before they reach the opposite side of the room 4 m away. The more diffuse corona products may also be the reason why the pattern observed with the experimental ionizer (Section 3.5) was not observed in these *Ionic Breeze*^{TM} exposure tests.

It is likely that the other allergens (Der f1, Der p2 and Fel d1), found to be destroyed by corona discharge in a pin-to-plane corona electrode configuration, will be susceptible to damage by the corona products produced by ionizers. Therefore, ionizers appear to present a novel method for destroying a number of different types of allergens in the domestic environment.

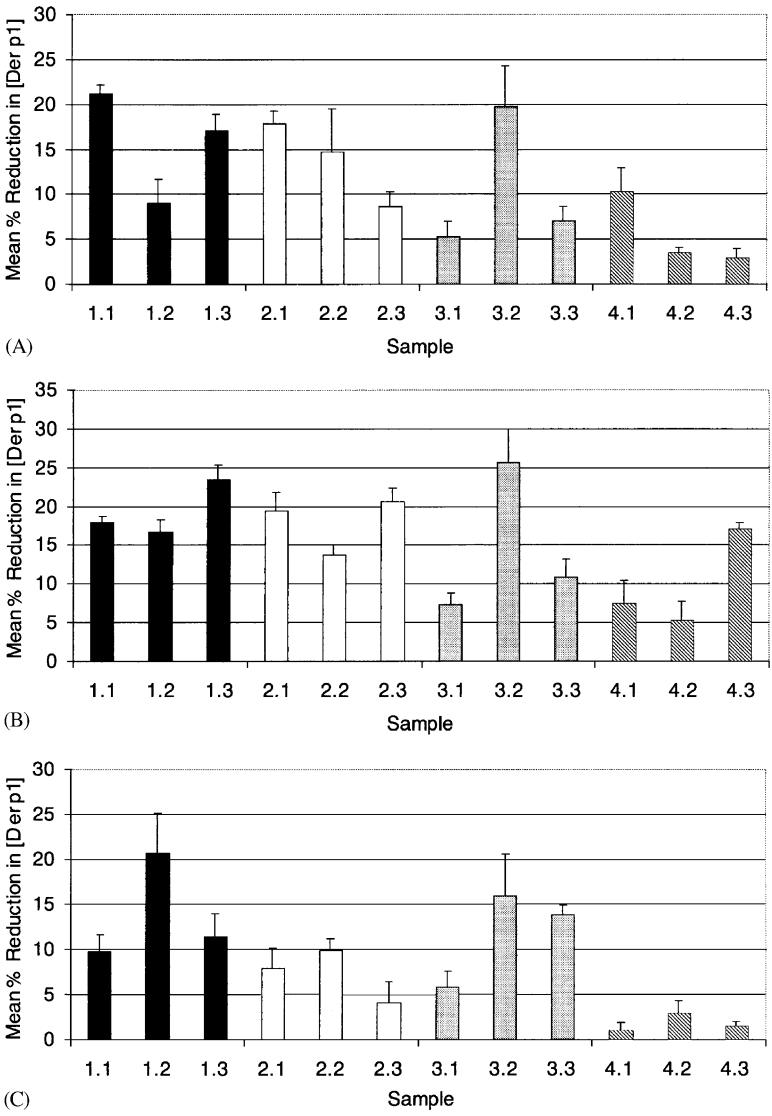

Fig. 9. The mean percentage reductions in Der p1 concentration of samples in various positions around the furnished room after a (A) 1 week exposure, (B) 2 week exposure, or (C) 3 week exposure to the *Ionic Breeze*™ *Silent Air Purifier*. The sample numbers refer to the position in each sector as shown in Fig. 3. All values are statistically significant ($n = 6$; SEM shown).

4. Discussion

Little data is, at present, available on the actual molecular consequences of corona discharge on proteins. However, corona discharges are widely used industrially as

chemical reactors for the surface treatment of polymers and data has been collected on the reactions of the corona products with these polymers [8]. Some of the bonds encountered in polymers, and affected by corona products are also present in proteins. These include the carboxyl group (C–OOH), which will be present at the carboxy terminus of the protein at the very least, and the CO and NH bonds, which constitute the peptide bond (CONH), present between every single amino acid residue. During corona discharge treatment of polymers, the above bonds are broken proportional to the extent 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 the emission of CO, CO_2 and H_2 molecules from the surface [23].

A similar reaction mechanism may also explain the exponential growth to maximum relationship of the percentage reduction in allergen concentration exhibited by all allergens (except Fel d1 after positive corona) when subjected to corona discharge. The maximum percentage reduction plateau may represent the saturation point where reactions were counterbalanced or otherwise limited. 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 have been destroyed and there would be no possibility of the protein renaturing. Another similarity with the industrial use of corona is that the efficiency of corona treatment depends upon the polarity of the corona. In this investigation, negative corona had a greater efficacy for destroying domestic allergens (except Der p2) than positive corona and this polarity is also generally more efficient for oxidation and etching [24].

One study that investigated the molecular changes that occurred after the exposure of proteins to corona discharge found that the disulphide bond between cysteine residues was oxidized to form two molecules of cysteic acid [25]. This is similar to the chemical reduction of the disulphide bridges of Group 1 that led to a more linear conformation [26]. As all allergens tested in these experiments contain three disulphide bonds [27–29], which in the mite allergens are known to stabilize the conformational epitopes, this reaction of cysteine to cysteic acid may also be responsible for the loss of binding to the antibodies used in ELISA.

The ELISA results indicated an alteration in the epitopes, which prevented the monoclonal antibody from binding. However, as human IgE may recognize different epitopes, the current investigation does not demonstrate that the epitopes used by human IgE are also corona-sensitive. Further studies are warranted to determine whether the destruction of allergens observed in these experiments also affect the epitopes recognized by human IgE.

At the present moment it would be unwise to recommend continuous use of ionizers in the domestic environment due to the fact that exposure to ozone is harmful—particularly to atopic individuals [30,31]. The tests described in this present paper have shown that molecular ozone is not the corona product responsible for the destruction of allergen. Therefore, methods of increasing the production of active species whilst keeping ozone production at a minimum would

be beneficial before any practical application of corona discharge to remove allergens from a variety of sources in the domestic environment is envisaged. Utilizing the optimal corona current for allergen destruction would also have the added benefit of reducing the associated safety risks by only using the necessary applied voltage in the appliance.

It has been shown that corona products from ionizers are effective at destroying vertically positioned Der p1 samples, although 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 [32]. The deposition of Fel d1 on walls is due to the particular 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 it has been reported that significant amounts of mite and cat allergen can be found on hair and clothing [33,34]. This has led to contamination of homes without cats, schools and other public places and caused significant amounts of allergen to be deposited [35]. Placing an ionizer close to garments would lead to the allergen carried on that garment to be destroyed, thereby reducing the risk of inter-building contamination.

The extent of destruction of surface allergens observed in the experiments reported here would not be reproduced with allergens not present on surfaces; other protocols would have to be developed in order to destroy allergen present deep within soft furnishings, mattresses, etc. Preliminary experiments have been performed into determining the extent of corona product penetration through different fabrics and thickness of open cell, reticulated foam and closed cell, expanded polypropylene foam used in soft furnishings [36]. 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 5 mm of closed cell foam and 49% and 44% less reduction was observed with 5 and 10 mm of open cell foam respectively. This shows that the simple method of placing an ionizer so that the ion wind is directed onto the soft furnishing would not be sufficient to significantly reduce the allergen reservoir within.

The potential application of using ionizers to reduce the allergen load within soft furnishings in the domestic environment would have to be more sophisticated. 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.

It has now been demonstrated that ionizers could be used to implement a clinical benefit to atopic individuals. If protocols could be developed in order to expose domestic allergens to the active corona products, whilst minimizing exposure to

patients, or pets, corona discharge could represent a safe, economic and simple process to reduce a patient's allergen exposure.

Acknowledgements

The support of the British National Asthma Campaign, who funded this work, is gratefully acknowledged.

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